PHYLOGENY, TAXONOMY, CHARACTER EVOLUTION, AND BIOGEOGRAPHY OF GLASSFROGS (AMPHIBIA: CENTROLENIDAE)

by

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ABSTRACT

The present study provides a new hypothesis of the evolutionary relationships of Glassfrogs (Centrolenidae) inferred from mitochondrial and nuclear loci, and addresses questions on the evolution and speciation of the group. Ingroup sampling includes 55.5% of the named taxa in the family, representing most of the phenotypic diversity described for the group. As outgroups, I included 35 species, most of which represent families traditionally associated with Glassfrogs. Gene sampling consisted of complete or partial sequences of three mitochondrial (12S, 16S, ND1) and three nuclear markers (*c-myc* exon 2, RAG1, POMC) for a total of ~4362 bp. Phylogenies were estimated using maximum parsimony, maximum likelihood, and Bayesian analyses for individual genes and combined datasets. The importance of analyzing mitochondrial and molecular datasets separately is discussed, with particular emphasis on the ways in which this approach clarifies interpretations of relationships within Glassfrogs and other Neotropical anurans.

Based on the phylogeny obtained, I propose a new taxonomy of Glassfrogs and its sister taxon *Allophryne ruthveni*. This arrangement formalizes clades that have significant statistical support under Parsimony, Maximum Likelihood, and Bayesian inference criteria, and that, in most cases, are phenotipically diagnosable. Centrolenid diversity is arranged into two subfamilies and a total of 12 genera, seven of which are new.

Using Maximum Likelihood and Parsimony, I explore the evolution of morphological and behavioral features that characterize this unique group of

Neotropical anurans. Each trait that has been postulated to trace relationships unambiguously in this group turns out to have had a complex evolutionary history with multiple origins and losses. Complete ventral transparency has evolved multiple independent times under all models of reconstruction, even those biased toward single origins. I demonstrate that repeated evolution of transparency has a significant inverse correlation across phylogeny with the presence of iridophores, which are hypothesized to protect internal organs from detrimental effects of solar radiation and heat. Complex derived behaviors, such as deposition of eggs on undersides of leaves, have evolved at least four times, and may be correlated with evolution of parental care. The evolution of sexually dimorphic traits (i.e., humeral spines) is ambiguous and depends on the method of inference; however, a scenario with recurrent origins of spines, probably selected in response to male-male intraspecific competition, is favored. The effect of incomplete sampling on reconstruction of ancestral character states is considered by comparing topologies and patterns of character evolution derived from analyses of complete and pruned datasets. Given the results, I suggest that when working with relatively large groups (i.e., $\geq$ 90 species), complete taxon sampling may not be so critical for an accurate reconstruction of character evolution, as long as morphological/behavioral diversity of all major clades is represented.

The biogeography of centrolenid frogs is partially resolved. Glassfrogs originated in South America and dispersed multiple times into Central America. The most likely scenario for the current distribution of the Centrolenidae suggests a Guianan origin with subsequent dispersal into the Amazonia and the Chocó. Once the

land connection between South and Central America was complete, and before the uplift of the Eastern Andean Cordillera, Glassfrogs dispersed to Central America at least four times. The uplift of the Eastern Cordillera and the Mérida Andes, coupled with drops in temperature during Pleistocene climatic fluctuations, could have facilitated dispersal of cold-adapted species from the Guianas to the Cordillera de la Costa and then into the Northern Andes. Glassfrogs reached the Southern Andes via repeated dispersal from the Northern Andes. Finally, comparisons of phylogeny and species distributions strongly supports vicariance as the main speciation mechanism in centrolenid frogs.

A mis taitas, mis ñaños y sobretodo a mi Elisa de mi vida

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INTRODUCTION

The anuran family Centrolenidae Taylor, 1951, is a monophyletic group nested within the Neobatrachia (Ruíz-Carranza and Lynch, 1991; Ford and Cannatella, 1993; Darst and Cannatella, 2004; Wiens et al., 2005; Frost et al., 2006; but see Haas, 2003). Currently, it contains 145 recognized species distributed throughout the Neotropics (southern Mexico to Bolivia, northeastern Argentina, and southeastern Brazil; updated from AmphibiaWeb, 2006). Glassfrogs are nocturnal, epiphyllous, and arboreal. All species have a partially or completely transparent venter, and deposit their eggs out of the water on vegetation (leaves or branches) overhanging streams or on rocks above streams (Ruíz-Carranza and Lynch, 1991).

Traditionally, Centrolenidae has been thought to be closely related to Hylidae (Lynch, 1973; Duellman, 1975, 2001; Ford and Cannatella, 1993) because frogs of both families have an intercalary element between the ultimate and penultimate phalanges; additionally, several species of Glassfrogs and hylids have green bones and a white ventral parietal peritoneum. However, several molecular and morphological studies (Noble, 1931; Austin et al., 2002; Burton, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006) indicate a Centrolenidae–Allophrynidae sister relationship.

Within the Centrolenidae, at present, the most widely accepted taxonomy of the group is that proposed by Ruíz-Carranza and Lynch (1991), who recognized the genera *Centrolene, Cochranella,* and *Hyalinobatrachium,* and several infrageneric species groups. Since the seminal paper by Ruíz-Carranza and Lynch (1991a),

several authors have proposed changes to the generic (Savage, 2002; Cisneros-Heredia and McDiarmid 2007) and infrageneric (Ruíz-Carranza and Lynch, 1995a, 1998; Duellman and Señaris, 2003; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006) groupings of species; these taxonomic modifications have been made based on one or few morphological characters and lack rigorous phylogenetic analysis. Although several authors (Noonan and Harvey, 2000; Frost et al., 2006; Guayasamin et al., 2006) have questioned the taxonomy proposed by Ruíz-Carranza and Lynch (1991a), no alternate hypotheses based on comprehensive phylogenetic analyses have been presented.

In the first chapter of this dissertation, I infer the evolutionary relationships of centrolenid frogs using mitochondrial and nuclear markers under maximum likelihood, Bayesian, and maximum parsimony criteria. Also, I test the validity of previously recognized genera and assess the relationship between Glassfrogs and other anuran families. In Chapter 2, I provide a taxonomy that is consistent with the recovered evolutionary history of Glassfrogs in the framework of the traditional nomenclatural system (ICZN, 1999), but that is also compatible with the PhyloCode (Cantino and de Quieroz, 2006). In Chapter 3, I explore the evolution of morphological and behavioral features that characterize centrolenid frogs and assess the effect of incomplete sampling on the reconstruction of ancestral character states. Finally, in Chapter 4, I discuss the origin and speciation patterns of Glassfrogs based on a phylogenetic framework and distribution of species.

CHAPTER 1

PHYLOGENETIC RELATIONSHIPS OF GLASSFROGS (CENTROLENIDAE) BASED ON MITOCHONDRIAL AND NUCLEAR GENES

Anurans of the family Centrolenidae are a monophyletic group nested within Neobatrachia (Ruiz-Carranza and Lynch, 1991a; Ford and Cannatella, 1993; Darst and Cannatella, 2004; Wiens et al., 2005; Frost et al., 2006; but see Haas, 2003). Currently, 147 species of Glassfrogs are recognized (AmphibiaWeb, 2006), distributed throughout the Neotropics. Centrolenids are and are nocturnal, epiphyllous, and arboreal; they have partially or completely transparent venters, and deposit their eggs on vegetation (leaves or branches) overhanging streams or on rocks above streams (Ruiz-Carranza and Lynch, 1991a).

To date, the most widely accepted taxonomy of the group is that of Ruiz-Carranza and Lynch (1991a, 1995a, 1998), who recognized the genera *Centrolene, Cochranella,* and *Hyalinobatrachium,* and several infrageneric species groups. Their generic classification was based on the presence of two morphological characteristics—humeral spines in adult male *Centrolene*, and a white, bulbous liver in *Hyalinobatrachium*—and the absence of both of these features in frogs of the genus *Cochranella.* This arrangement implies that the evolutionary patterns of these derived characters (i.e., humeral spines and bulbous, white liver) are unequivocal, and that the frogs and the characters share a perfectly congruent evolutionary history. However, recent research has revealed a surprising amount of evolutionary lability in

morphological traits previously thought to be conservative (e.g., Parra-Olea and Wake, 2001; Wiens et al., 2003; Mueller et al., 2004); the results of these studies suggest that phylogenies based solely on morphological characters should be tested with independent datasets. Several authors (Frost et al., 2006; Guayasamin et al., 2006) have questioned the monophyly of the groups proposed by Ruiz-Carranza and Lynch (1991a), but no alternate hypotheses based on comprehensive phylogenetic analyses have been proposed. Herein, I present a molecular hypothesis of the relationships of Glassfrogs based on multiple independent loci. This comprehensive phylogeny is intended to provide a new evolutionary context for studies addressing the biology and systematics of this fascinating group of tropical anurans.

MATERIALS AND METHODS

Taxonomy and terminology

Throughout this work, I use the name Centrolenidae as originally presented by Taylor (1951; i.e., exclusive of *Allophryne ruthveni*). When referring to the current taxonomy of centrolenid frogs, I follow the generic and infrageneric classifications as proposed by Ruiz-Carranza and Lynch (1991a, 1995a, 1998), with the addition of the genus *Nymphargus* (Cisneros-Heredia and McDiarmid, 2007). Family and generic placement of outgroups are as summarized by Frost (2007), except for the placement of *Allophryne ruthveni,* for which I maintain the use of Allophrynidae (Guayasamin and Trueb, 2007). Museum abbreviations follow Frost (2007), with the following

additions: CBG = Centro de Biodiversidad y Genética, Cochabamba, Bolivia; CH = Círculo Herpetológico, Panamá; MHUA = Museo de Herpetología de la Universidad de Antioquia, Colombia; MIZA = Museo del Instituto de Zoología Agrícola Francisco Fernández Yépez, Venezuela; MHNC = Museo de Historia Natural Cusco, Universidad Nacional de San Antonio Abad del Cusco. Abbreviations for field series of individuals are as follow: AAV = Alvaro Andres Velasquez; CFBH = Célio F. B. Haddad; BPN = Brice P. Noonan; DFCH-USFQ = Diego F. Cisneros-Heredia, Universidad San Francisco de Quito, Ecuador; IDLR: Ignacio De la Riva; KHJ = Karl-Heinz Jungfer; MAD = Maureen A. Donnelly; MAR = Marco Rada; NRPS = Nely Rocio Pinto; MB = Michel Blanc.

Ingroup and outgroup taxon sampling

I obtained molecular data for 80 recognized and 10 undescribed centrolenid species (Appendix 1.1). The ingroup sampling thus represents 55.5% of the known species diversity of Centrolenidae, including representatives from all currently recognized genera and infrageneric groups, and all the major ecoregions in which these anurans occur.

Traditionally, amphibian systematists have considered Centrolenidae to be closely related to Hylidae (Duellman, 1975, 2001; Ford and Cannatella, 1993; Lynch, 1973) because frogs of both families have an intercalary element between the ultimate and penultimate phalanges. Additionally, several species of Glassfrogs and hylids have green bones and a white ventral parietal peritoneum. However, recent studies

based on molecular and/or morphological data (Austin et al., 2002; Burton, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006) support the hypothesis that the monotypic Allophrynidae is the sister species of Centrolenidae. Other groups proposed to be closely related to Centrolenidae are Leptodactylidae, Dendrobatidae, and Bufonidae (Biju and Bossuyt, 2003; Darst and Cannatella, 2004; Heinicke et al., 2007; Roelants et al., 2007). In the analyses, I include 35 species to represent all clades that have been associated with centrolenid frogs (Appendix 1.2). I used *Xenopus laevis* and *Spea bombifrons* as more distant outgroups to root the phylogeny.

Data collection

Tissue samples were obtained from specimens listed in Appendix 1.1. Additional sequences were downloaded from GenBank (NCBI; Appendix 1.2). I included relatively fast evolving mitochondrial loci for resolution of recent divergences, as well as more slowly evolving nuclear loci to illuminate relationships among older clades. The genes chosen for this study are the mitochondrial 12S rRNA, 16S rRNA, NADH Dehydrogenase Subunit 1 (ND1), and the nuclear proto-oncogene cellular myelocytomatosis (*c-myc*), proopiomelanocortin A gene (POMC), and recombination activating gene 1 (RAG1).

Genomic DNA was extracted from frozen, Lairds buffer, or ethanol-preserved tissues with the DNeasyTissue extraction kit (Qiagen Inc.) or using standard phenol-chloroform extraction protocols (Sambrook et al., 1989). Primers and PCR

amplification protocols are presented in Tables 1.1 and 1.2, respectively. PCR products were visualized in agarose gels, and unincorporated primers and DNTPs were removed from PCR products using ExoSap purification (ExoSap-it, GE Healthcare). Cycle sequencing reactions were completed using the corresponding PCR primers and BigDye Terminator 3.1 chemistry (Applied Biosciences), with a standard cycle sequencing profile (96°C/3 min; 35 cycles of 96°C/10 s, 50°C/15 s, 60°C/3 min; and 72°C/7 min). Reaction products were purified with CleanSEQ magnetic beads (Agencourt) and run in an ABI Prism 3100 Genetic Analyzer (Applied Biosciences) or purified using ethanol precipitation and run in an ABI 3730xl. Data from heavy and light strands were compared to generate a consensus sequence for each taxon using Sequencher 4.1 (Gene Codes Corp., 2000). Sequences were initially aligned in CLUSTAL_X (Thompson et al., 1997) and adjusted by hand in MacClade ver. 4.07 (Maddison and Maddison, 2000); manual adjustments were particularly important in protein coding genes to maintain reading frames. In some cases (*Centrolene altitudinale, C. prosoblepon, C. venezuelense, Cochranella granulosa, C. oyampiensis, Hyalinobatrachium* aff. *mondolfii;* Table 1.3), incomplete sequences from different individuals of the same species were joined to construct a single complete composite sequence for the combined analyses to reduce the number of terminal taxa and simplify search space. I applied this approach after confirming that the genetic distances between the shared DNA fragments were minimal (nucleotide divergence < 1%). GenBank accession numbers for all individual

sequences generated in this study are listed in Appendices 1.1 and 1.2. All alignments will be available at TreeBase once this study is published.

Phylogenetic analysis

Phylogenetic analyses were conducted using maximum parsimony (MP), maximum likelihood (ML), and Bayesian analyses (BA) for individual genes, as well as for a combined dataset. Parsimony analyses were performed in PAUP*4b10 (Swofford, 2002) using heuristic searches (10,000 stepwise random additions with TBR branch-swapping) and clade support was estimated via 500 bootstrap pseudo-replicates with 10 random additions (Felsenstein, 1985). ML analyses were run in RAxML (Randomized Axelerated Maximum Likelihood for High Performance Computing 2.2.0; Stamatakis, 2006; available at <http://icwww.epfl.ch/~stamatak/index-Dateien/Page443.htm>), which uses a GTR + CAT (GTR with per-site rate categories) as an approximation to GTR + Γ, and allows for data partitioning (Stamatakis, 2006), a feature that has not been implemented in other likelihood programs. I performed a total of 100 runs to reduce the probability of inferring a suboptimal likelihood solution. Node support was assessed via 1000 bootstrap replicates, partitioning the dataset by gene (12S, 16S) or by gene and codon position in protein coding genes (ND1, *cmy-c,* POMC, Rag1, and combined datasets).

For BA analyses, I implemented the model of nucleotide substitution selected as the best fit for every particular dataset (partition) according to the Akaike Information Criterion (AIC) in ModelTest version 3.7 (Posada and Crandall, 1998;

Table 1.3). Bayesian analyses of each mitochondrial and nuclear gene and the combined datasets were performed in Mr Bayes 3.1 (Ronquist and Huelsenbeck, 2003). The combined datasets were analyzed partitioning the data by gene (12S, 16S, ND1, *cmy-c*, POMC, RAG1) and nucleotide position (first, second, and third positions for ND1, *cmy-c*, POMC, and RAG1). The analysis for each gene consisted of a minimum of 5 million generations and four Markov chains with default heating values. The prior used for the rate matrix was a uniform dirichlet and no prior information on topology was incorporated. Trees were sampled every 1000 generations; stationarity was assessed by examining the standard deviation of split frequencies and by plotting the –ln L per generation using Tracer Version 1.2.1 (Rambaut and Drummond, 2003–2005), and trees generated before stationarity were discarded as "burn-in." For the combined dataset, runs were as described above, but consisted of 20 million generations. Two independent runs were performed for the combined mitochondrial, combined nuclear, and complete dataset to assess if the resulting topologies and posterior probabilities were congruent.

Topological congruence and combinability

Topologies resulting from each gene were compared to detect areas of incongruence that are strongly supported by non-parametric bootstrap values and/or posterior probabilities (Wiens, 1998). I did not employ the Incongruence Length Difference (ILD) test as it has been shown to be a poor check of the compatibility of separate data partitions (Hipp et al., 2004). Bootstrap values $\geq$ 70% are considered to

indicate strong support (Hillis and Bull, 1993, with their caveats). Clades with posterior probabilities ≥ 0.95 are considered strongly supported, but I caution that relatively high posterior probabilities for short internodes (particularly those with low bootstrap values) may be over-estimates of confidence (Alfaro et al., 2003; Erixon et al., 2003).

Statistical testing of alternative phylogenies

Probabilistic approaches to testing phylogenetic hypotheses include parametric ML tests (Goldman et al., 2000; Hulsenbeck and Bull, 1996; Hulsenbeck et al., 1996; Swofford et al., 1996), nonparametric ML tests (Shimodaira and Hasegawa, 1999), and Bayesian posterior probabilities (Huelsenbeck and Ronquist, 2001; Larget and Simon, 1999; Li et al., 2000; Mau et al., 1999; Rannala and Yang, 1996; Yang and Rannala, 1997). Buckley (2002) demonstrated that parametric ML tests tend to produce Type-I errors because of model misspecification coupled with branch-length heterogeneity, a result also mentioned by Huelsenbeck et al. (1996). In contrast, the Shimodaira-Hasegawa (SH) test was observed to be much more conservative, even under high substitution rate and branch-length heterogeneity (Buckley, 2002). The SH-test takes multiple comparison corrections into consideration and allows evaluation of *a priori* and *a posteriori* hypotheses (Goldman et al., 2000; Shimodaira and Hasegawa, 1999), but it also requires simultaneous comparison of all reasonable topologies to ensure that the true topology is available for any bootstrap data set (Goldman et al., 2000). Buckley (2002) suggested that the

number of candidate topologies should be minimized through the application of prior knowledge. In the case of this study, restricting the set of possible topologies to those that represent prior taxonomic hypotheses is relatively simple (Fig. 1.1). However, incorporating all the possible topologies that are compatible with these *a priori* hypotheses was impractical given the large number of species. With this limitation in mind, I used the complete dataset and searched for the best tree compatible with each prior hypothesis (Fig. 1.1) with the program RAxML. Then, I performed a SH test including the best tree as estimated by RAxML and the best trees compatible with the following prior hypotheses: (1) Monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991a: Fig. 1.1A); (2) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella*, monophyletic *Centrolene* + *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991a; as modified by Bolívar et al., 1999; Fig. 1.1B); (3) monophyletic *Centrolene*, monophyletic *Cochranella*, monotypic *Teratohyla* (sensu Taylor, 1949, 1951; Fig. 1.1C); (4) monophyletic *Centrolenella* with three species groups, monotypic *Centrolene* (sensu Savage, 1967; Fig. 1.1D); (5) monophyletic *Centrolene*, monophyletic *Centrolenella*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991a; modified by Savage, 2002; Fig. 1.1E); (6) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved *Cochranella*, with their respective species groups (sensu Ruiz-Carranza and Lynch, 1991a, 1995a, 1998; Fig. 1.1F); and (7) monophyletic *Centrolene*, monophyletic *Hyalinobatrachium*, unresolved

Cochranella, with their corresponding species groups (sensu Ruiz-Carranza and Lynch, 1991a, 1995a, 1998; modified by Duellman and Señaris, 2003; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006a, b, 2007; Fig. 1.1G).

From a Bayesian perspective, I interpreted support for the alternate hypotheses (Fig. 1.1) based on posterior probabilities. From all possible trees found during the MCMC search, the probability of a particular hypothesis being correct was calculated as the proportion of the trees in agreement with the hypothesis, using the filter command in PAUP* with a constraint describing the hypothesis.

RESULTS

Molecular data and models of evolution

For most of the species in Centrolenidae, I obtained a total of ~4362 bp from the following markers: mitochondrial 12S rRNA (~974 bp), fragment of mitochondrial 16S rRNA (~895 bp), mitochondrial ND1 (~973 bp), nuclear POMC (~934 bp), nuclear *c-myc* exon 2 (~430 bp), and nuclear RAG1 (~456 bp). See Appendix 1.1 for genes sequenced for each individual. Parameter value estimates for best-fit models for each gene and codon position are summarized in Table 1.3. As expected, mitochondrial genes presented more variability across taxa than nuclear genes (Table 1.4). For some data partitions in the ML analysis, I used a slightly more parameter-rich model than that estimated in ModelTest 3.7 (Table 1.3) because of constraints of the software used to perform the ML analysis (RAxML). However, I expect this modification to have little influence on the topology (Kelchner and

Thomas, 2007).

Relationships of Glassfrogs and other anurans

For the individual genes, analyses recovered congruent topologies using parsimony, ML, and Bayesian criteria. In Bayesian analyses, multiple runs produced almost identical topologies and posterior probabilities. Given that no strongly supported conflicts were observed when comparing individual gene trees, I proceeded to combine the datasets. The resulting mitochondrial and nuclear topologies are shown in Figures 1.2 and 1.3. Multiple runs of the complete dataset produced different likelihood values (Fig. 1.4), from which the topology with the best score was chosen (Fig. 1.5).

When analyzed separately, most of the genes have limited ability to recover ancient relationships. Three genes (16S, ND1, and *cmy-c*) are unable to resolve relationships among families. The remaining genes (12S, RAG1, POMC) show support, although without reaching statistical significance, for an evolutionary affinity between Centrolenidae and *Allophryne ruthveni*, a relationship that becomes significant in the combined analyses (Fig. 1.2). The affinities of other anurans to the Centrolenidae + *Allophryne* clade are poorly resolved, though the topology inferred from the complete dataset places *Leptodacylus didymus* (family Leptodactylidae) as a close relative (Fig. 1.2). Other interesting results include the recovery of a clade consisting of all marsupial frogs sampled, which recently were split into three families (Amphignathodontidae, Cryptobatrachidae, Hemiphractidae) by Frost et al.

(2006). Finally, I consistently recover a clade that is compatible with Hyloidea as defined by Darst and Cannatella (2004).

Relationships within Centrolenidae are congruent among individual genes. When combining genes, five well-defined clades are inferred (Figs. 1.3, 1.5). However, a noticeable incongruence emerges when comparing nuclear and mitochondrial topologies. The mitochondrial phylogeny suggests a Clade A + B, whereas the nuclear tree places Clade A as sister to Clade C (Fig. 1.3). In the gene-by-gene analyses, only POMC was found to support the clade A + C. The other nuclear genes either weakly supported A + B (*c-myc*), or did not resolve the relationships between these clades (RAG1). Each of the mitochondrial genes inferred an A + B clade with non-significant support.

The only conspicuous uncertainty is the phylogenetic position of *Centrolene tayrona* (Clade D). None of the genes places this species in a clade with strong support. For example, two nuclear genes (POMC, RAG1) suggest an affinity between *C. tayrona* and species in Clade B. In contrast, the other nuclear gene (*cmy-c*) shows some support for *C. tayrona* as the sister species of all other Glassfrogs. The mitochondrial genes are ambiguous for the placement of the species as well. The combined datasets place *C. tayrona* as an early divergent species, but fail to resolve its relationship with other clades (Figs. 1.3, 1.5).

Statistical testing of alternative phylogenies

The nonparametric SH test rejects all previous hypotheses of centrolenid relationships (Fig. 1.1) when compared to the best tree ($P < 0.001$); additionally, none of the previous hypotheses is represented in the ".trprobs" file generated during the MCMC analyses of the complete dataset, implying that their Bayesian posterior probability is close to zero.

DISCUSSION

Relationships of Glassfrogs and other anurans

The relationships between centrolenid frogs and other anurans are partially resolved in my analyses. I consistently inferred a monophyletic Centrolenidae + *Allophryne ruthveni* clade with mitochondrial and nuclear genes (Fig. 1.2). This relationship is not a surprise. It was first proposed by Noble (1931) and since then, has been corroborated by several morphological (Burton, 1998, 2004; da Silva, 1998; Duellman, 2001; Wiens et al., 2005), as well as molecular (Austin et al., 2002; Faivovich et al., 2005; Frost et al., 2006; Grant et al., 2006; Wiens et al., 2005) characters. Frost et al. (2006) proposed a rearrangement of the Linnaean ranks to formalize the *Allophryne* + Centrolenidae clade. They recognized the family Centrolenidae with two subfamilies (i.e., Allophryninae and Centroleninae). Frost et al. (2006) proposal has the virtue of naming a natural group. On the other hand, if it is accepted, it has an obvious drawback—it will disassociate decades of literature and

the names of the merged families. Alternate solutions to avoid taxonomic instability include the use of unranked names (Cantino and de Queiroz, 2007) or using other family-group ranks (e.g., subsuperfamily; ICZN, 1999). A forthcoming work on the taxonomy of Glassfrogs will address the pending issue of the name for the *Allophryne*-Centrolenidae clade (Chapter 2).

At deeper nodes, the mitochondrial topology fails to resolve relationships, a result that is expected given the rapid evolution of mtDNA (Brown et al., 1979). The nuclear genes suggest that leptodactylid frogs are close relatives of the *Allophryne* + Centrolenidae clade, in agreement with Frost et al. (2006). Also inferred by the nuclear topology is a clade that contains families (i.e., Allophrynidae, Brachycephalidae, Centrolenidae, Ceratophryidae, Dendrobatidae, Hemiphractidae, Leptodactylidae); all but Allophrynidae, Ceratophryidae, and Leptodactylidae have derived reproductive strategies (i.e., eggs placed out of water and different levels of parental care). The monophyly of this clade implies an ancient origin of a derived reproductive mode that subsequently diversified into the various modes observed in the extant species (Duellman and Trueb, 1994, and references therein). However, given the low support for the clade, I refrain from further speculation and recommend more thorough investigation with additional nuclear genes.

Another noteworthy result is the inference of a monophyletic group that contains all sampled marsupial frogs (*Flectonotus, Hemiphractus, Gastrotheca, Stefania*). This clade is recovered with significant support in the nuclear (Bayesian and maximum likelihood criteria) and complete (Bayesian criterion) phylogenies, but

not in the mitochondrial topology (Fig. 1.2). It has been shown that analysis of fast-evolving genes (e.g., mitochondrial genes) produces phylogenies with relatively poor resolution among old lineages when compared to those inferred from nuclear genes, probably because the high mutation rate of mitochondrial DNA leads to homoplasy, obscuring phylogenetic signal (Brown et al., 1979; Swofford et al., 1996, and references therein). Given that the monophyly of marsupial frogs has been inferred in previous molecular studies (Wiens et al., 2005; Wiens et al., 2007) and is supported by morphological and life history traits (Burton, 2004; Duellman and Maness, 1980; Wassersug and Duellman, 1984; Wiens et al., 2005), I recommend that Amphignathodontidae and Cryptobatrachidae recently recognized by Frost et al. (2006) be placed in the synonymy of Hemiphractidae Peters 1862, as suggested by Wiens et al. (2007). The example of marsupial frogs illustrates the benefits of analyzing nuclear and mitochondrial genes separately before combining datasets.

Phylogenetic relationships within Centrolenidae

The phylogeny of Glassfrogs contains five main clades (Clades A–E) as inferred from the mitochondrial, nuclear, and complete datasets (Figs. 1.3, 1.5). The mitochondrial and nuclear topologies are congruent except for the position of Clade A. As mentioned above, the sister relationship between A + C that is recovered in the combined nuclear tree is only supported by one of the three nuclear genes (POMC), whereas c-myc favors an A + B clade, and RAG1 does not provide resolution. It is obvious then, that the combined nuclear topology is influenced mostly by the POMC

dataset. In contrast, all mitochondrial genes consistently inferred an A + B clade, which is also supported with statistical significance by the complete dataset (Fig. 1.5). Although the data at hand do not allow us to reach a definite conclusion about which of the arrangements is correct, at the moment I favor the hypothesis of a Clade A + B. The observed incongruence among nuclear genes could be originated by the effect of stochastic lineage sorting (Tajima, 1983; Neigel and Avise, 1986; see McCracken and Sorenson, 2005). If a rapid radiation originated Clades A, B, and C, stochastic lineage sorting may produce incongruence of individual gene trees with the history of speciation. In such cases, because coalescence time is directly related to effective population size (N_e), and the mitochondrial N_e is about one-quarter that of any nuclear locus, the probability of coalescence is greater for the mitochondrial genome than it is for a nuclear gene (Moore, 1995), in other words, mitochondrial genes are more likely to recover the species tree.

The phylogeny (Fig. 1.5) is highly incongruent with previous hypotheses of centrolenid relationships based on phenetics or phenotypic phylogenetics (Taylor, 1949; Savage, 1967, 2002; Ruiz-Carranza and Lynch, 1991a; Cisneros-Heredia and McDiarmid, 2007) and not on the principles of phylogenetic systematics and homology (Hennig, 1966; Patterson, 1982; Wiley, 1981). I found that none of the genera that has been proposed for the family are monophyletic, although some of the species groups correspond to natural groups (discussed below).

At present, the most commonly accepted classification of Glassfrogs is that of Ruiz-Carranza and Lynch (1991a, 1995a, 1998), who proposed an arrangement built

on two putative synapomorphies (humeral spine in males of the genus *Centrolene;* white, bulbous liver in species of *Hyalinobatrachium*). The merit of this proposal is that emphasizes the use of synapomorphies in the classification, although it requires an unambiguous evolution of these two characters. The results presented in this chapter reveal a more complex scenario in which similarity is a product of convergence and/or parallelism, contradicting the hypotheses presented by Ruiz-Carranza and Lynch (1991a, 1995a, 1998). This work on Glassfrogs corroborates the idea that morphological homoplasy is not a rare phenomenon (Bossuyt and Milinkovitch, 2000; Parra-Olea and Wake, 2001; Wiens et al., 2003, 2006), and that hypotheses of relationships based on few traits are likely to misrepresent true phylogeny. This point does not imply that morphology is phylogenetically uninformative; it only means that derived characters have had more than one origin in Glassfrogs. For example, humeral spines are prevalent in Clade A, and a white, bulbous liver is present in all species of Clade E (except *Cochranella vozmedianoi* and *C. revocata*), which perfectly corresponds to the *Hyalinobatrachium fleischmanni* species group (Ruiz-Carranza and Lynch, 1991a; Savage, 1967). However, these traits are not exclusive in the aforementioned clades, highlighting the importance of congruence as the mechanism to test hypotheses of homology (de Pinna, 1991; Patterson, 1982).

Last, the novel hypothesis of relationships of Glassfrogs presented herein opens numerous venues of research that need to be addressed in future studies. The interpretation of character evolution in Glassfrogs needs reevaluation, especially

regarding the origin of similar morphologies in distantly related species (e.g., bulbous liver, humeral spine, complete ventral transparency). Other questions that remain to be answered include: What are the main processes that have driven the speciation of Glassfrogs? Is parental care correlated with the probability of diversification/extinction of lineages? Do sister species share similar ecological niches? Equally important is the need of developing a taxonomy that is congruent with the inferred phylogeny.

CHAPTER 2

PHYLOGENETIC TAXONOMY OF GLASSFROGS (ANURA: CENTROLENIDAE) AND ITS SISTER TAXON *ALLOPHRYNE RUTHVENI*

Our classifications will come to be, as far as they can be so made, genealogies

Darwin (1859).

On Chapter 1, I presented a novel hypothesis of the evolutionary relationships of Glassfrogs (Figs. 1.5) and commented on the differences between it and previous hypotheses of relationships (Ruiz-Carranza and Lynch, 1991a, 1995a, 1998; Savage, 2002; Cisneros-Heredia and McDiarmid, 2007). Also, I showed that the inferred phylogeny is significantly better than alternatives trees. The main goal of this study is to provide a classification that is congruent with the inferred phylogeny. Also, I review briefly the taxonomic history of Glassfrogs and the hypotheses of relationships between them and other anurans.

Centrolenidae, its monophyly and relationships with other anurans

Jiménez de la Espada (1872) described the first genus and species of Glassfrog, *Centrolene geckoideum,* and placed it the family Polypedatidae (now a synonym of Rhacophoridae). It was not until the work of Taylor (1951) that the family Centrolenidae was proposed, defined mainly by the distal limb bones, the tibiale (astragalus) and fibulare (calcaneus), being wholly or partially fused in its

members. Since then, the monophyly of Glassfrogs has not been questioned. Additional morphological synapomorphies include dilated medial process on Metacarpal III (Hayes and Starrett, 1980), T-shaped terminal phalanges (Taylor, 1951), intercalary element between distal and penultimate phalanges (Taylor, 1951), complete or partial fusion of tibiale and fibulare (Taylor, 1951; Sanchiz and De la Riva, 1993), eggs deposited out of water (Ruiz-Carranza and Lynch, 1991a), and ventral parietal peritoneum partially or completely transparent (Fig. 2.1). All of these characters occur in other anuran clades (reviewed by Cisneros-Heredia and McDiarmid, 2006a, 2007), but there is consensus among amphibian researchers that these similarities result from convergent evolution rather than common ancestry. Several authors (e.g., Ruiz-Carranza and Lynch, 1991a; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006a; Guayasamin and Trueb, 2007) have pointed out the fact that the dilated medial process on Metacarpal III (Hayes and Starrett, 1980) seems to be the most useful morphological characteristic to differentiate Glassfrogs from other anurans. Additional putative synapomorphies may include myological (Burton, 1998, 2004; da Silva, 1998; Señaris and Ayarzagüena, 2005), osteological (Guayasamin and Trueb, 2007), chromatic (Schwalm and McNulty, 1980), and larval traits (Haas, 2003); however, their relevance remains to be tested at a general scale.

Molecular phylogenies have supported the view of centrolenids as a natural group. Several independent studies based on different datasets and methods have shown high levels of congruence at retrieving a monophyletic Centrolenidae (e.g.,

Darst and Cannatella, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1).

The relationship of Glassfrogs with other anurans has remained controversial. After Jiménez de la Espada (1872) considered that *Centrolene geckoideum* was related to *Rhacophorus,* and Taylor (1951) suggested a close affinity with Heleophryninae, most researchers associated centrolenids with Hylidae and Pseudidae based on the presence of intercalary elements (Lynch, 1974; Duellman and Trueb, 1994; Ford and Cannatella, 1993; Rueda-Almoacid, 1994). Using larval morphology, Haas (2003) suggested Centrolenidae as sister group of Neobatrachia except *Limnodynastes.* In a molecular study, Darst and Cannatella (2004) explicitly rejected a Centrolenidae + Hylidae + Pseudidae clade, and presented evidence supporting a Centrolenidae + Leptodactylidae clade (see also Roelants et al., 2007). Contradicting this hypothesis, Biju and Bossuyt (2003) favored Centrolenidae as sister taxon of Bufonidae + Dendrobatidae, although with low support.

Several studies have inferred a close affinity between Centrolenidae and *Allophryne ruthveni* Gaige 1926, a hypothesis first mentioned by Noble (1931). Morphological (Duellman, 2001; Burton, 2004; Wiens et al., 2005) and molecular (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1) studies have corroborated Allophrynidae as the sister taxon of Centrolenidae. This led Frost et al. (2006) to rank this clade as the family Centrolenidae, containing the subfamilies Allophryninae and Centroleninae. This classification has been followed in some works (e.g., Cisneros-Heredia and

McDiarmid, 2006a, 2007; Castroviejo-Fisher et al., 2007), but Guayasamin and Trueb (2007) stated that the proposal led to unnecessary taxonomic instability, an argument that I expand herein (see Results and Discussion).

Phylogenetic studies that include in their sampling *Allophryne* and Centrolenidae show total congruence in placing *Allophryne* as the sister taxon of Glassfrogs (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1), but moderate congruence about the placement of this clade with respect to other anurans (Frost et al., 2006; Grant et al., 2006; Chapter 1). Given that each study is limited in one aspect or another (e.g., taxon and genes sampling), it is difficult to interpret the real significance of these differences. Then, based on the mentioned results, it is fair to say that the close evolutionary relationships between *Allophryne ruthveni* and Centrolenidae is well established, but that the relationships of this clade with other frogs are uncertain (Fig. 1.2).

Taxonomic history of Centrolenidae

The grouping of species of Glassfrogs into genera and infrageneric categories has been complex and unstable mainly because hypotheses of relationships have been based on the arbitrary application of a few characters and the lack of cladistic analyses (see proposals by Savage, 2002; Cisneros-Heredia and McDiarmid, 2007). In this section, I summarize in chronological order the main studies that focus on the systematics of centrolenid frogs. I exclude from this review papers with species descriptions, which are listed in Appendix 2.2, but mention extensive reviews of

species and/or characters. Taxonomically relevant characters in Glassfrogs are illustrated in Figures 2.2, 2.3, and 2.4.

- Jiménez de la Espada (1872).—Description of *Centrolene geckoideum*, type species for the genus *Centrolene,* characterized by the presence of vomerine teeth and humeral spine in males. Jiménez de la Espada considered *C. geckoideum* to be related to rhacophorid frogs.
- Noble (1920).—Description of the genus *Centrolenella* (type species *C. antioquiensis*) characterized by lacking vomerine teeth and humeral spines. Noble commented on similarities and differences of *Centrolenella* and *Centrolene;* additionally, he suggested the possibility of including *Hyla prosoblepon* Boettger 1892 in *Centrolenella* (formally assigned by Noble [1924]) and the placement of both genera (*Centrolene* and *Centrolenella*) in Leptodactylidae.
- Dunn (1931).—Placement of *Centrolenella* under the synonymy of *Centrolene*.
- Taylor (1949).—Recognition of *Centrolene* for species in which males had humeral spines. Species lacking spines were assigned to the genus *Centrolenella*.
- Taylor (1951).—Description of the family Centrolenidae and recognition of three genera: *Centrolene* for species in which males have humeral spines (*Centrolenella* is a synonym); *Teratohyla* (type species: *Centrolenella spinosa* Taylor 1949) for species with prepollical spines and lacking humeral spine;

Cochranella (type species: *Centrolenella granulosa* Taylor 1949) for species lacking both humeral and prepollical spines. Taylor (1951) diagnosed Centrolenidae based, mainly, on complete fusion of the tibiale and fibulare, T-shaped terminal phalanges, and the presence of an intercalary cartilage between penultimate and ultimate phalanges. Taylor considered centrolenids to be related to the African frogs in Heleophrynidae.

- Goin (1964).—Recognition of *Centrolenella* and a monotypic *Centrolene.* Goin characterized *Centrolene* by its large body size, diameter of the disc of Finger III wider than that of the eye, and a developed humeral spine in males.
- Savage (1967).—Followed Goin's arrangement and placed Central American Glassfrogs in *Centrolenella.* Within this genus, Savage proposed three species groups: *fleischmanni* Group for species with white bones, white dorsal coloration in preservative, transparent parietal peritoneum, white hepatic and visceral peritonea, and absence of humeral spine and vomerine teeth; *prosoblepon* Group for species with green bones, lavender dorsal coloration in preservative, white (= partially white) parietal peritoneum, transparent hepatic peritoneum, and presence or not of humeral spines in males and vomerine teeth; *pulverata* Group for species having green bones, white dorsal coloration in preservative, transparent parietal peritoneum, white hepatic and visceral peritonea, absence of humeral spine in males but presence of vomerine teeth.
- Rivero (1968).—Revision of the centrolenid frogs of Venezuela following the generic proposal suggested by Goin (1964).

- Starrett and Savage (1973).— Addition of liver shape (bulb-shaped/bulbous vs. trilobed) and egg coloration (green vs. dark) as taxonomically important characters. These authors also mention that species in the *fleischmanni* Group usually deposit green eggs on the under surfaces of leaves overhanging streams.
- Lynch and Duellman (1973).—Revision of the centrolenid frogs of Ecuador following the generic proposal suggested by Goin (1964) and the infrageneric groups of Savage (1967). Additionally, these authors presented a review of taxonomic characters.
- Hayes and Starrett (1980).—Description of a dilated medial process on Metacarpal III as a diagnostic character for Centrolenidae. The validity of this character as synapomorphy of Centrolenidae is well established; to date, it has been observed in 71 species (Guayasamin and Trueb, 2007).
- Lynch (1981).—Association of the name *Hylopsis platycephalus* Werner 1894 with a centrolenid species from the Sierra Nevada de Santa Marta in Colombia, making *Centrolenella* a synonym of *Hylopsis*.
- McDiarmid and Savage (1984).—Rejection of the association of *Hylopsis platycephalus* with a centrolenid species from the Sierra Nevada de Santa Marta in Colombia. These authors argued that Lynch (1981) forced *Centrolenella* into *Hylopsis* by assuming that the original description of *H. platycephalus* by Werner was incorrect. They also mentioned that the type and only known specimen of *H. platycephalus* is lost, precluding a resolution of

this problem, and that to preserve nomenclatural stability the name *Centrolenella* should be conserved.

- Rivero (1985).—Revision of the Venezuelan centrolenid species, with their placement into three groups within the genus *Centrolenella*. The *andina* Group included species with humeral spines in males, Finger I shorter than II, presence of vomerine teeth (not in all species), lavender dorsal coloration in preservative and presence of dark spots in dorsum (not in all species). The *pulidoi* Group was diagnosed by lacking humeral spine in males, having a Finger I shorter than II, presence of vomerine teeth, a well defined canthus rostralis together with a vertical loreal region, reduced webbing between fingers, and dark brown dorsal coloration in preservative. The *fleischmanni* Group included species lacking humeral spines and vomerine teeth, and having a Finger I shorter or longer than II, white dorsal coloration in preservative, transparent parietal peritoneum, and white visceral peritonea.
- Ruiz-Carranza et al. (1986).—Rediagnosis of the genus *Centrolene*. These authors added black egg coloration as a characteristic of *Centrolene*.
- Ruiz-Carranza and Lynch (1991a).—Proposal of a new generic classification explicitly based on the principle of synapomorphy. This seminal work provided a testable hypothesis of relationships among centrolenids. The classification was based on two putative synapomorphies and divided Glassfrogs in three genera: (1) *Centrolene* was considered monophyletic, with *Centrolenella* as a synonym, being the presence of a humeral spine in adult

males its only synapomorphy. This genus was subdivided into three phenetic groups. The *geckoideum* Group contains species with small eyes, green bones, trilobate liver covered by transparent peritoneum, white parietal peritoneum, white parietal pericardium, and vomerine teeth. The *prosoblepon* Group includes species with large eyes, green bones (white in *Centrolene tayrona* Ruiz-Carranza and Lynch, 1991b), trilobate liver covered by transparent peritoneum, white parietal peritoneum, white pericardium, visceral peritoneum white or transparent, vomerine teeth present or absent. The *peristictum* Group included species with large eyes, pale green bones, trilobate liver covered by transparent peritoneum, white visceral peritoneum, white pericardium, and absence of vomerine teeth. (2) *Hyalinobatrachium* was considered as a monophyletic genus. Ruiz-Carranza and Lynch (1991a) hypothesized that the presence of a bulbous liver covered by white peritoneum was an unambiguous synapomorphy. The genus was divided in three phenetic groups: *fleischmanni* Group for species with large eyes, white bones in life, white visceral peritonea, white or transparent pericardium, and no vomerine teeth; *parvulum* Group containing species with large eyes, green or white bones, white peritoneum covering the urinary bladder and heart, and vomerine teeth present; *pulveratum* Group, large eyes, pale green bones, white pericardium and visceral peritonea, vomerine teeth present. (3) Species that lacked humeral spines and a white bulbous liver were placed in the genus *Cochranella*, which Ruiz-Carranza and Lynch (1991a) considered a non-

monophyletic group. Initially, two phenetic groups were recognized with *Cochranella*: the *granulosa* Group for species with large eyes, pale green bones, three-lobed liver, white pericardium, parietal and visceral peritonea, and vomerine teeth present; and the *ocellata* Group, for species with large eyes, bones from green to white, trilobate liver, white parietal peritoneum [transparent in *C. ametarsia* (Flores, 1987)], vomerine teeth present or absent.

- Ruiz-Carranza and Lynch (1991b, 1995a, 1998).—Redefinition of the infrageneric classification presented in 1991. Most species in the *Cochranella granulosa* Group were characterized as having sloping snouts in lateral view, protruding upper lip, and fleshy ulnar and tarsal folds. The former *Cochranella ocellata* Group was split into two groups: a newly defined *Cochranella ocellata* Group, with reduced webbing between external fingers added to the previous characters and the new *Cochranella spinosa* Group, with the same characters than the former *ocellata* Group, but with moderate to extensive webbing between external fingers. The *Hyalinobatrachium fleischmanni* Group was proposed as a clade based on a putative synapomorphy: eggs held in a single layer on the undersides of leaves. Furthermore, this Group was divided into the *fleischmanni* Subgroup with white pericardium, and the *chirripoi* Subgroup with transparent pericardium.
- Bolívar et al. (1999).—Addition of combat behavior between males as a character to resolve intergeneric relationships. These authors proposed that an elaborate combat behavior is a synapomorphy that clusters *Centrolene* and

Cochranella (sensu Ruiz-Carranza and Lynch, 1991a). In this behavior, first described by Duellman and Savitzky (1976), males fight dangling upside down while holding the vegetation by their hind legs, grasping one another venter-to-venter (Fig. 2.3). In contrast, all *Hyalinobatrachium* males are hypothesized to have an amplexus-like fighting behavior, which was considered to be primitive. Further observations of the derived behavior have been reported by Guayasamin & Barrio-Amorós (2005) and Kubicki (2007).

- Savage (2002).—Generic-level modifications of the classification by Ruiz-Carranza and Lynch (1991a). Savage limited the genus *Centrolene* to species in the *geckoideum* Group sensu Ruiz-Carranza and Lynch (1991a), and resurrected *Centrolenella* for the remaining Glassfrogs that have humeral spines. Also, this book includes species accounts for all Costa Rican Glassfrogs.
- Duellman and Señaris (2003).—Erection of the *gorzulai* Group and modification of the diagnosis of *Hyalinobatrachium*. The *gorzulai* Group was diagnosed, mainly, by having a white hepatic peritoneum and males with a small humeral spine. Other characters defining the group included large eyes, green bones, parietal peritoneum reduced to the area covering the heart, white or transparent visceral peritoneum, and vomerine teeth absent. This rendered *Hyalinobatrachium* defined by a sole unambiguous synapomorphy, the presence of a bulbous liver.

- Señaris and Ayarzagüena (2005).—Review of Venezuelan Glassfrogs and erection of the *Cochranella oyampiensis* Group. The main characteristics in the *oyampiensis* Group are the absence of humeral spines and the presence of a white hepatic peritoneum; other characters include large eyes, green or pale green in life, white parietal peritoneum (reduced to the anterior quarter), white pericardium, and white visceral peritonea.
- Frost et al. (2006).—Placement of Glassfrogs and *Allophryne ruthveni* in a single family, Centrolenidae. Glassfrogs were placed into the subfamily Centroleninae; the monotypic subfamily Allophryninae contained *A. ruthveni*. The arrangement by Frost et al. (2006) was disputed by Guayasamin and Trueb (2007) and is discussed in detail below. Additionally, Frost et al. (2006) questioned the monophyly of *Centrolene* and *Cochranella* (sensu Ruiz-Carranza and Lynch, 1991a), although their taxon sampling was limited (4 species).
- Cisneros-Heredia and McDiarmid (2006a).—Modifications at the infrageneric level and comments on genera and characters. These authors introduced several changes: redescription of the *gorzulai* Group as having complete or almost complete transparent peritoneum, and a trilobated or bulbous liver; re-allocation of the *pulveratum* Group into the genus *Cochranella*; and fusion of the *peristictum* Group with the *prosoblepon* Group. Also, they reviewed the characters supporting each of the genera and phenetic groups and concluded

that the only monophyletic unit was the *fleischmanni* Group, although without analyzing the data in a cladistic framework.

- Guayasamin et al. (2006b).—Questioning of the monophyly of generic and infrageneric grouping in Centrolenidae. Based on a cladistic analysis of morphological and behavioral characters, these authors argue that only the *fleischmanni* Group could be seen with confidence as a monophyletic unit. Also, they summarize the current classification of Glassfrogs excluding the changes of Savage (2002) and Cisneros-Heredia and McDiarmid (2006a).
- Cisneros-Heredia and McDiarmid (2007).—Description of the genus *Nymphargus,* comprehensive study of the morphological and behavioral characters of Glassfrogs, and generic and infrageneric redefinitions. These authors elevated the *Cochranella ocellata* Group (except *Cochranella balionota* [Duellman, 1981] and *Cochranella ocellata* [Boulenger, 1918]) to the rank of genus under the name *Nymphargus.* Also, they eliminated the *geckoideum* Group and the *chirripoi* Subgroup. Species previously assigned to the *parvulum* Group were considered as *incertae sedis* within Centrolenidae. In Appendix II, Cisneros-Heredia and McDiarmid (2007) provided a summary of the classification of Centrolenidae (including Allophryninae) with the proposed modifications.
- Kubicki (2007).—Review of the Glassfrogs of Costa Rica, with emphasis on their biology.

- Kok and Castroviejo-Fisher (in press)—Transferred *Cochranella oyampiensis* (Lescure, 1975) to the *spinosa* Group, and renamed the former *oyampiensis* Group as the *castroviejoi* Group.
- Finally, in Chapter 1, I provided a molecular phylogeny of centrolenid frogs. I provided the first extensive molecular phylogeny of centrolenid frogs using mitochondrial and nuclear genes. This phylogeny differs significantly from previous morphology-based hypotheses of relationships and is the base for the taxonomic proposal presented below.

MATERIALS AND METHODS

Taxonomy and Terminology

Throughout this work, I use the name Centrolenidae as originally intended by Taylor (1951), exclusive of *Allophryne ruthveni*. An alternative taxonomy can be found in Frost et al. (2006). When referring to the current taxonomy, I follow the generic and infrageneric classifications as proposed by Ruiz-Carranza and Lynch (1991a, 1995a, 1998), with the addition of the recently described *Nymphargus* Cisneros-Heredia and McDiarmid, 2007. Fingers are numbered preaxially to postaxially from I–IV to facilitate comparison with previous literature dealing with anurans. However, I stress that in an evolutionary perspective, anuran fingers should be numbered from II–V, consistent with the hypothesis that Digit I was lost in anurans (Shubin and Alberch, 1986; Fabrezi and Alberch, 1996). Osteological terminology is that of Duellman and Trueb (1994), Fabrezi (1992, 1993), and Trueb

(1973). Morphology of nuptial excrescences is as described by Flores (1985) and Cisneros-Heredia and McDiarmid (2007), with the modification presented in Figure 2.4. Museum abbreviations follow those of Frost (2007). Material examined is listed in Appendix 2.1.

Phylogenetic framework for the new classification

The phylogeny inferred in Chapter 1 included ~55% of the recognized taxa in Centrolenidae and 35 outgroups. Gene sampling consisted of complete or partial sequences for the following markers: mitochondrial 12S rRNA (~974 bp), fragment of mitochondrial 16S rRNA (895 bp), mitochondrial ND1 (~973 bp), nuclear POMC (~634 bp), nuclear *c-myc* (~430 bp), and nuclear RAG1 (~456 bp). Below, I discuss the clades shown in Figures 1.3 and 1.5, but also incorporated the relevant results obtained from the gene by gene analyzes, which were not discussed in detailed in Chapter 1. The sequences and alignments of the mentioned genes are available at TreeBase and GenBank, respectively. Previous taxonomic hypotheses of centrolenid frogs (Taylor, 1949, 1951; Savage, 1967, 2002; Ruiz-Carranza and Lynch, 1991a, 1995a, 1998; as modified by Bolívar et al., 1999; Duellman and Señaris, 2003; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006a, 2006b, 2007) were rejected by parametric tests when compared to the topology shown in Figure 1.1.

Criteria for naming taxa

When naming taxa, I adhere to the International Code of Zoological Nomenclature (ICZN, 1999), a system based on the use of the binomen and hierarchical categories, first established by Linnaeus (1758). Also, for each named taxon, I provide a phylogenetic characterization compatible with the phylogenetic definition required by the PhyloCode (Cantino and de Queiroz, 2007). The recognition of supraspecific taxa as ranks is arbitrary and taxa with the same ranks may not be comparable (unless they are sister taxa). Thus, to reduce subjectivity in associating clades with ranks, I followed the following criteria:

1. Significant statistical support and congruence among phylogenetic estimation methods. I name clades supported by significant values under Parsimony, Maximum Likelihood, and Bayesian criteria (Fig. 1.5). For Parsimony and Maximum Likelihood, bootstrap values $\geq 70\%$ are considered to indicate strong support (Hillis and Bull, 1993, with their caveats). In a Bayesian framework, clades with posterior probabilities ≥ 0.95 are considered strongly supported, but with the caveat that relatively high posterior probabilities for short internodes (particularly those with low bootstrap values) may be over-estimates of confidence (Erixon et al., 2003; Alfaro et al., 2003).
2. Congruence among genetic markers (genes). When analyzed independently, there is no strongly supported incongruence among loci (Wiens et al., 2005). This does not mean that all genes inferred a particular clade with significant support; it only indicates that there is no conflicting signal from independent markers.

3. Morphological and/or behavioral distinctiveness. I favor naming clades that present phenotypic synapomorphies or at least a combination of characters that allow species assignation into those particular clades.
4. Traditional use of names. When previously recognized groups are recovered as monophyletic or nearly monophyletic, the new classification minimizes the number of name changes required to make these groups monophyletic, preserving, when possible, the names and contents of the most generally accepted previous classification.

Briefly, the *modus operandi* was to identify phylogenetically stable clades (criteria 1 and 2), identify which of these clades were possible to diagnose by phenotypic characters (criteria 3), and try to minimize the number of new names (criteria 4). The methodology has the ultimate goal to provide a long-term stability in the names of Glassfrogs.

RESULTS

A monophyletic taxonomy

Based on the topology presented in Figure 1.5 and on the criteria described in the previous section, I propose a revised taxonomy that is congruent with the evolutionary history of Glassfrogs and their closest relative, *Allophryne ruthveni.* A list of all currently recognized centrolenid species with their previous generic placements and names proposed herein is provided in Appendix 2.2. Several species

are considered as *incerta sedis* because molecular data are not available, and phenotypic characters are not sufficient to place them into monophyletic genera with confidence (Appendix 2.2).

This proposal formalizes the close affinity between centrolenid frogs and *Allophryne ruthveni*, maintaining the use of the names Allophrynidae and Centrolenidae (see Frost et al., 2006 for an alternative classification). Within Centrolenidae, I recognize two subfamilies and a total of 12 genera, seven of which are new.

SUBSUPERFAMILY: **Centrolenia** Jiménez de la Espada, 1872

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolene geckoideum* Jiménez de la Espada, 1872, and *Allophryne ruthveni* Gaige, 1926.

DIAGNOSIS: Some of the most conspicuous nonmolecular characters hypothesized to be shared by *Allophryne ruthveni* and centrolenid frogs include presence of T-shaped terminal phalanges (Duellman, 2001), ventral process on terminal phalanges of fingers (da Silva, 1998), *m. flexor digitorum brevis superficialis* of the foot inserts with two tendons (Burton, 2004). Additional morphological characters supporting Centrolenia can be found in Burton (1998, 2004), da Silva (1998), Duellman (2001), and Wiens et al. (2005). Molecular studies supporting the validity of this clade include Austin et al. (2002), Faivovich et al. (2005), Wiens et al. (2005), Frost et al. (2006), Grant et al. (2006), and Chapter 1.

CONTENT: Centrolenidae Taylor, 1951, and Allophrynidae Goin, Goin, and Zug, 1978.

ETYMOLOGY: The name Centrolenia is derived from the genus *Centrolene;* the suffix *–ia* means "having the nature of." The name Centrolenia formalizes the clade Centrolenidae + Allophrynidae.

DISTRIBUTION: Tropical Mexico to Bolivia and northeastern Argentina, and in southeastern Brazil, with highest diversity in the northern Andes.

COMMENTS: Phylogenetics studies including *Allophryne*, Centrolenidae, Hylidae and Leptodactylidae show total congruence in placing *Allophryne* as the sister taxon of Glassfrogs (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1). Recently, Frost et al. (2006) merged Centrolenidae Taylor, 1951, and Allophrynidae Goin, Goin, and Zug, 1978, into a single family (i.e., Centrolenidae); if accepted, this action immediately disassociates decades of literature and the names of the merged families. Frost et al. (2006) intended to formalize the clade Centrolenidae + Allophrynidae; I assume that Frost et al. (2006) decided to reorganize the available ranks because a superfamily incorporating these two families, among others, already exists (i.e., Hyloidea Rafinesque, 1815; sensu Dubois, 2005), even though this produced nomenclatural instability. Following the principles of ICNZ (1999), name instability should be avoided when possible; therefore I follow an alternative course. Article 35.1 of the ICZN states: "The family group encompasses all nominal taxa at the ranks of superfamily, family, subfamily, tribe, subtribe, and any other rank below superfamily

and above genus that may be desired." Thus, creating the Subsuperfamily Centrolenia formalizes the evolutionary proximity of Centrolenidae and Allophynidae, avoids nomenclatural instability (Fig. 2.5), and fulfills the primary principle of zoological nomenclature (i.e., maintenance of name stability; ICZN, 1999).

FAMILY: **Allophrynidae** Goin, Goin, and Zug, 1978

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon consisting of *Allophryne ruthveni* Gaige, 1926, and other species that share a more recent common ancestor with *A. ruthveni* than with *Centrolene geckoideum* Jiménez de la Espada, 1872.

TYPE GENUS: *Allophryne* Gaige, 1926.

DIAGNOSIS: Nonmolecular characteristics present in *Allophryne ruthveni* and absent in centrolenid frogs include: eggs deposited in water (Duellman, 1975), tibiale and fibulare not fused (Fabrezi and Langone, 2000), dilated medial process on Metacarpal III absent, neopalatine absent. Additional characteristics of *Allophryne ruthveni* can be found in Lynch and Freeman (1966), Burton (1998, 2004), and Fabrezi and Langone (2000).

CONTENT: *Allophryne ruthveni* Gaige, 1926.

SISTER TAXON: Centrolenidae Taylor, 1951. Noble (1931) was the first to associate *Allophryne ruthveni* with centrolenid frogs. Morphological (Duellman, 2001; Burton, 2004; Wiens et al., 2005) and molecular (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1) data

corroborate the Allophrynidae + Centrolenidae clade.

DISTRIBUTION: *Allophryne ruthveni* is known from the Guianan region of South America (Venezuela, Guyana, Suriname, French Guianan to north central Brazil) and central Brazil at elevations of 0–300 m (Langone and Segalla, 1997; Caldwell and Hoogmoed, 1998; Fig. 2.6).

COMMENTS: Savage (1973) considered *Allophryne ruthveni* to form a monotypic family (Allophrynidae), but neither described nor diagnosed the family. The first diagnosis of Allophrynidae is that of Goin et al. (1978). Frost et al. (2006) considered this taxon as a subfamily (Allophryninae); for reasons explained above, I do not follow their suggested taxonomy.

GENUS: ***Allophryne*** Gaige, 1926

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon nested within Allophrynidae that includes *Allophryne ruthveni* Gaige, 1926.

TYPE SPECIES: *Allophryne ruthveni* Gaige, 1926, by original designation.

DIAGNOSIS: Same as Allophrynidae Goin, Goin, and Zug, 1978.

CONTENT: *Allophryne ruthveni* Gaige, 1926.

DISTRIBUTION: Same as the family Allophrynidae (see above).

FAMILY: **Centrolenidae** Taylor, 1951

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of extant Glassfrogs.

TYPE GENUS: *Centrolene* Jiménez de la Espada, 1872.

DIAGNOSIS: Nonmolecular synapomorphies present in all centrolenid frogs include: dilated medial process on Metacarpal III (Hayes and Starrett, 1980); intercalary element between distal and penultimate phalanges (Taylor, 1951); complete or partial fusion of tibiale and fibulare (Taylor, 1951; Sanchiz and De la Riva, 1993); eggs deposited out of water (Ruiz-Carranza and Lynch, 1991a); and ventral parietal peritoneum partially or completely transparent (Fig. 2.1). Additional putative synapomorphies include myological (Burton, 1998, 2004; da Silva, 1998; Señaris and Ayarzagüena, 2005), osteological (Guayasamin and Trueb, 2007), chromatic (Schwalm and McNulty, 1980), and larval (Haas, 2003) traits.

CONTENT (12 GENERA): *Celsiella* new genus; *Centrolene* Jiménez de la Espada, 1872; *Chimerella* new genus; *Cochranella* Taylor, 1951; *Espadarana* new genus; *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991a; *Ikakogi* new genus; *Nymphargus* Cisneros-Heredia and McDiarmid, 2007; *Rulyrana* new genus; *Sachatamia* new genus; *Teratohyla* Taylor, 1951; *Vitreorana* new genus (Figs. 2.7, 2.8, 2.9).

SISTER TAXON: Allophrynidae Goin, Goin, and Zug, 1978. Morphological (Burton, 2004; Duellman, 2001; Wiens et al., 2005) and molecular (Austin et al., 2002; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1) evidence suggests that Allophrynidae is the sister taxon of Centrolenidae. Noble (1931) first associated *Allophryne ruthveni* with centrolenids based on similarities of internal and external morphology.

DISTRIBUTION: Tropical Central America, tropical Andes, Sierra Nevada de Santa Marta in Colombia, Cordillera de la Costa of Venezuela, Guianan Shield, Amazon Basin, and Atlantic Forest of Brazil; highest diversity in northern Andes (Fig. 2.6).

COMMENTS: I use the family name Centrolenidae as originally intended by Taylor (1951). Frost et al. (2006) applied the name Centrolenidae to the clade that defined herein as Centrolenia. I consider that the terminology presented herein reflects the current understanding of evolutionary relationships, and, at the same time, maintains the historic association between the name Centrolenidae (sensu Taylor, 1951) and its literature. The topologies inferred using the combined mitochondrial, combined nuclear, and complete datasets (Figs. 1.2), consistently recovered a monophyletic Centrolenidae. The monophyly of Centrolenidae is further supported by morphological (Duellman, 2001; Burton, 2004; Wiens et al., 2005) and other molecular (Austin et al., 2002; Darst and Cannatella, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Chapter 1) data. The placement of species in the family Centrolenidae is unambiguous because, as far as I know, this is the only Neotropical clade with a medial process on Metacarpal III (see Guayasamin and Trueb, 2007; Fig. 2.1).

SUBFAMILY: **Centroleninae** Jiménez de la Espada, 1872

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolene geckoideum* Jiménez de la Espada,

1872, and *Cochranella granulosa* Taylor, 1949.

TYPE GENUS: *Centrolene* Jiménez de la Espada, 1872.

DIAGNOSIS: A characteristic that diagnoses the subfamily Centroleninae is the presence of a relatively long prepollex (relative length > 50% of Metacarpal I), which is short (< 50% of Metacarpal I) in Hyalinobatrachinae; however, more observations are necessary to confirm the validity of this trait. Additionally, most species in Centroleninae have lobed livers (tri-, tetra-, or penta-), green bones in life [white bones in *Nymphargus rosadus* (Ruiz-Carranza and Lynch, 1997); Rada, unpublished data], and a lavender dorsum in preservative. Males call from the upper surfaces of leaves, and females of most species deposit their eggs on the upper surfaces of leaves; exceptions where females place egg clutches on the undersides of leaves include *Centrolene peristictum* (Lynch and Duellman, 1973), *C. notostictum* Ruiz-Carranza and Lynch, 1991b, *Teratohyla spinosa* Taylor, 1949, *Sachatamia albomaculata* (Taylor, 1949), and *Vitreorana eurygnatha* (Lutz, 1925) (M. Bustamante, pers. comm.; Ruiz-Carranza and Lynch, 1991b; Starrett, 1960; McCranie and Wilson, 2002; Lutz, 1947; respectively). Humeral spines have evolved multiple times within Centroleninae, but they are completely absent in Hyalinobatrachinae.

CONTENT (9 GENERA): *Centrolene* Jiménez de la Espada, 1872, *Chimerella* new genus, *Cochranella* Taylor, 1951, *Espadarana* new genus, *Nymphargus* Cisneros-Heredia and McDiarmid, 2007, *Rulyrana* new genus, *Sachatamia* new genus, *Teratohyla* Taylor, 1951, *Vitreorana* new genus.

SISTER TAXON: Uncertain. There is no resolution among the three early

divergent branches of Centrolenidae (Figs. 2.7, 2.8). Two taxa are the possible sister taxon of Centroleninae; these are *Ikakogi* new genus or Hyalinobatrachinae new subfamily.

DISTRIBUTION: Tropical Central America, tropical Andes, Cordillera de la Costa of Venezuela, Guianan Shield, Amazon Basin, and Atlantic Forest.

COMMENTS: The clade Centroleninae is inferred with statistical support by the combined dataset.

GENUS: ***Centrolene*** Jiménez de la Espada, 1872

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolene geckoideum* Jiménez de la Espada, 1872, and *Centrolenella peristicta* Lynch and Duellman, 1973.

TYPE SPECIES: *Centrolene geckoideum* Jiménez de la Espada, 1872, by monotypy.

DIAGNOSIS: I cannot identify any unambiguous, nonmolecular synapomorphies for this clade. Nevertheless, the following combination of characteristics is diagnostic of *Centrolene:* (1) humeral spines present in adult males of most species, except *Centrolene daidaleum* Ruiz-Carranza and Lynch, 1991c and *C. savagei* Ruiz-Carranza and Lynch, 1991c*;* (2) tri-, tetra-, or pentalobed liver, covered by a transparent hepatic peritoneum; (3) ventral parietal peritoneum white anteriorly and transparent posteriorly; (4) bones varying from pale to bright green in life; (5) dorsum lavender in preservative, with or without spots; (6) dorsum of males

with conspicuous spinules during breeding season; (7) nuptial pads conspicuous in males; (8) dentigerous process of the vomer having or lacking teeth; (9) males usually call from the upper sides of leaves and females deposit egg masses on the upper sides of leaves along streams, although *C. geckoideum* calls from behind waterfalls or near spray zones and deposits the eggs on rocks (Lynch et al., 1983; Rueda-Almonacid, 1994; Grant et al., 1998), and *C. peristictum* calls and deposits eggs from the undersides of leaves (M. R. Bustamante, pers. comm.); (10) fighting behavior unknown for most species, but in *C. buckleyi* (Boulenger, 1882), males dangle by their feet and grapple venter-to-venter (Bolívar et al., 1999); (11) tibiale and fibulare partially or completely fused; (12) quadratojugal articulating with maxilla. The presence of humeral spines in the adult males of species in *Centrolene* distinguishes this clade from most other genera (Fig. xx). The other two genera presenting this trait are *Espadarana* new genus and the monotypic *Ikakogi* new genus. *Ikakogi tayrona* is the only centrolenid in which females guard egg clutches (Rada, unpublished data). The morphological convergence between *Centrolene* and *Espadarana* is remarkable. At this point, I am not aware of any discrete morphological character that would differentiate species from the two clades. However, most species in *Centrolene* lack teeth on the vomers (teeth present in *C. geckoideum* and *C. savagei*), whereas all species in *Espadarana* have vomerine teeth. Clades that contain some species with small humeral spines, which are not homologous to the spines in *Centrolene*, include *Nymphargus, Cochranella*, and *Vitreorana* (Fig. 2.10).

CONTENT (20 SPECIES): *Centrolene altitudinale* (Rivero 1968), *C.*

antioquiense (Noble 1920), *C. bacatum* Wild 1994, *C. buckleyi* (Boulenger 1882), *C. daidaleum* (Ruiz-Carranza & Lynch 1991c) new combination, *C. geckoideum* Jiménez de la Espada 1872, *C. hesperium* (Cadle & McDiarmid 1990), *C. hybrida* Ruiz-Carranza & Lynch 1991b, *C. notostictum* Ruiz-Carranza & Lynch 1991b, *C. peristictum* (Lynch & Duellman 1973), *C. pipilatum* (Lynch & Duellman 1973), *C. savagei* (Ruiz-Carranza & Lynch 1991c) new combination, *C. venezuelense* (Rivero 1968). Based on morphology and distribution pattern, we tentatively place in *Centrolene* the following species*: C. gemmatum* (Flores 1985), *C. heloderma* (Duellman 1981), *Centrolene lynchi* (Duellman 1980), *C. paezorum* Ruiz-Carranza, Hernández-Camacho, & Ardila-Robayo 1986, *C. sanchezi* Ruiz-Carranza & Lynch 1991b, *C. scirtetes* (Duellman & Burrowes 1989), and *C. solitaria* (Ruiz-Carranza & Lynch 1991c) new combination. DISTRIBUTION: The genus *Centrolene* is restricted to the northern Andes, from the Cordillera de Mérida in Venezuela, across the Andes of Colombia and Ecuador, to the Cordillera de Huancambamba in northern Peru, at elevations of 1100–3100 m (Fig. 2.11).

SISTER TAXON: The sister relationship of *Centrolene* Jiménez de la Espada, 1872 and *Nymphargus* Cisneros-Heredia and McDiarmid, 2007 is supported by the mitochondrial dataset and the overall combined dataset (Figs. 2.7, 2.8). However, the combined nuclear topology supports a *Centrolene* + Clade C (Fig. 2.8). I favor the *Centrolene* + *Nymphargus* hypothesis because it results from a more complete analysis. Also, it seems that the topology obtained from the combined nuclear dataset mostly derives from the information contained in the gene POMC, which is the only

nuclear gene that supports *Centrolene* + Clade C. The other nuclear genes either show weak support for a *Centrolene* + *Nymphargus* clade (*c-myc*) or lack any resolution at this level (RAG1). Nevertheless, this should be taken with caution because the mtDNA dataset has more characters than the nuclear; besides, mitochondrial genes are more prone to homoplasy due to higher mutation rates, and introgression events leave a stronger signal in uniparental inherited markers.

COMMENTS: The genus *Centrolene* (*sensu* Ruiz-Carranza and Lynch, 1991a) was characterized by a single morphological feature, the presence of a humeral spine in adult males. However, as suggested by Frost et al. (2006) and corroborated by this study, coding this character as the presence or absence of a humeral spine is overly simplistic. Several species [e.g., *"Nymphargus" armatus* (Ruiz-Carranza and Lynch, 1996) *"Cochranella" balionota* (Duellman, 1981), and *Nymphargus griffithsi* (Goin, 1961)] have a conspicuously developed ventral humeral crest (Ruiz-Carranza and Lynch, 1991a; Lynch and Ruiz-Carranza, 1996), which is easily confused with a poorly developed humeral spine. Based on the inferred phylogeny, it seems that the evolution of humeral spines is complex. At least four genera (*Chimerella, Centrolene, Cochranella, Vitreorana*) contain species with conspicuous humeral spines; so this feature seems to have undergone multiple origins (or losses). A detailed consideration of patterns of morphological evolution among centrolenid frogs is beyond the scope of this paper and will be addressed in a subsequent study. A monophyletic *Centrolene* was inferred in analyzes of the RAG1, *c-myc*, POMC, combined nuclear, and complete datasets.

GENUS: ***Nymphargus*** Cisneros-Heredia and McDiarmid, 2007

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Cochranella cochranae* Goin, 1961, and *Centrolenella bejaranoi* Cannatella, 1980.

TYPE SPECIES: *Cochranella cochranae* Goin, 1961.

DIAGNOSIS: The most conspicuous characteristics of *Nymphargus* are the reduced webbing between the outermost (= postaxial) fingers (Fingers III and IV) and the absence of humeral spines in males, except for a small humeral spine in *N. grandisonae* (Cochran and Goin, 1970). The following character states diagnose *Nymphargus:* (1) humeral spines absent in males of most species (present in *N. grandisonae*); (2) tri- or tetralobed liver, covered by a transparent hepatic peritoneum; (3) ventral parietal peritoneum white anteriorly and transparent posteriorly; (4) bones green in life [white in *N. rosadus* (Ruiz-Carranza and Lynch, 1997)*;* Rada, unpublished data]; (5) dorsum lavender in preservative, with or without spots; (6) dorsum of males usually with conspicuous spinules during breeding season; (7) Type I nuptial pads conspicuous in reproductive males; (8) males call from the upper sides of leaves, and females deposit egg masses on the upper sides of leaves along streams; (9) fighting behavior unknown for most species, but in *N. griffithsi* and *N. ignota* (Lynch, 1990), males dangle by their feet and grapple venter-to-venter (Duellman and Savitsky, 1976; Restrepo-Toro, 1996); (10) tibiale and fibulare partially or completely fused.

The genera *Celsiella* and *Rulyrana* are morphologically similar to *Nymphargus.* However, species of *Celsiella* have a small prepollex (length < 50% of Metacarpal I; Fig. 2.12), and cream to gray dorsal coloration in preservative. Furthermore, *Celsiella* is restricted to the Cordillera de la Costa of Venezuela, whereas *Nymphargus* is endemic to the Andes. Species of *Rulyrana* have moderate to extensive webbing between Fingers III and IV (basal or absent in *Nymphargus;* Fig. 2.13).

CONTENT (33 SPECIES): *Nymphargus bejaranoi* (Cannatella 1980), *N. cochranae* (Goin 1961), *N. garciae* (Ruiz-Carranza & Lynch 1995a), *N. grandisonae* (Cochran & Goin 1970) new combination, *N. griffithsi* (Goin 1961), *N. megacheirus* (Lynch & Duellman 1973), *N. mixomaculatus* (Guayasamin, Lehr, Rodríguez & Aguilar 2006a), *N. pluvialis* (Cannatella & Duellman 1982), *N. posadae* (Ruiz-Carranza & Lynch 1995a), *N. puyoensis* (Flores & McDiarmid 1989) new combination, *N. rosada* (Ruiz-Carranza & Lynch 1997), *N. siren* (Lynch & Duellman 1973), *N. wileyi* (Guayasamin, Bustamante, Almeida-Reinoso & Funk 2006b). Species for which we lack molecular data, but that have a morphology that corresponds to the diagnosis (see above) are tentatively placed in *Nymphargus*. These species are: *N. anomalus* (Lynch & Duellman 1973), *N. armatus* (Lynch & Ruiz-Carranza 1996), *N. buenaventura* (Cisneros-Heredia & Yánez-Muñoz 2007), *N. cariticommatus* (Wild 1994), *N. chami* (Ruiz-Carranza & Lynch 1995b), *N. chancas* (Duellman & Schulte 1993), *N. cristinae* (Ruiz-Carranza & Lynch 1995b), *N. ignotus* (Lynch 1990), *N. laurae* Cisneros-Heredia & McDiarmid 2007, *N. luminosus* (Ruiz-

Carranza & Lynch 1995b), *N. luteopunctatus* (Ruiz-Carranza & Lynch 1996), *N. mariae* (Duellman & Toft 1979) new combination, *N. nephelophilus* (Ruiz-Carranza & Lynch 1991d), *N. ocellatus* (Boulenger ,1918) new combination, *N. oreonympha* (Ruiz-Carranza & Lynch 1991d), *N. phenax* (Cannatella & Duellman 1982), *N. prasinus* (Duellman 1981), *N. ruizi* (Lynch 1993), *N. spilotus* (Ruiz-Carranza & Lynch 1997), and *N. truebae* (Duellman 1976). As mentioned before, the placement of these species is tentative and should be tested with an independent source of characters (see Appendix 2.2).

SISTER TAXON: *Centrolene* Jiménez de la Espada, 1872. See comments on the Sister Taxon section below *Centrolene*.

DISTRIBUTION: *Nymphargus* is endemic to the Andes and Andean foothills in Colombia, Ecuador, Peru, and Bolivia. Most of the species are restricted to elevations > 1000 m, and only *N. cochranae* and *N. laurae* are found at lower elevations on the Amazonian slopes of the Andes (Fig. 2.11).

COMMENTS: Ruiz-Carranza and Lynch (1991a, 1995a) created the *Cochranella ocellata* Group for species that have reduced webbing between Fingers III and IV, lobed liver, visceral and hepatic peritonea without white iridophores, and that lacked humeral spines. The phylogenetic analysis shows that most of the species included in the *ocellata* Group are part of a natural group. Also, the reduced webbing between Fingers III and IV is a synapomorphy, although not unambiguous, of the clade. After a strictly phenetic analysis of morphological characters, Cisneros-Heredia and McDiarmid (2007) rearranged the generic classification of

Centrolenidae, placing most species of the *Cochranella ocellata* Group in a new genus, which they named as *Nymphargus.* This genus is paraphyletic and lacks the morphological cohesion of the *ocellata* Group sensu Ruiz-Carranza and Lynch (1991a, 1995a). For example, Cisneros-Heredia and McDiarmid (2007) excluded species (e.g., *C. ocellata, puyoensis, mariae*) that present all but one (color pattern) of the diagnostic characteristics that they listed for *Nymphargus*. Given that the most reliable synapomorphy of the genus is the reduced webbing between Fingers III and IV combined with the absence of humeral spines (see diagnosis), the species composition of *Nymphargus* (as defined herein) is more similar to the *ocellata* Group of Ruiz-Carranza and Lynch (1991a, 1995a) than *Nymphargus* sensu Cisneros-Heredia and McDiarmid (2007). The clade *Nymphargus* is well supported by 12S, 16S, ND1, *c-myc,* POMC, RAG1, combined mitochondrial, combined nuclear, and complete datasets.

GENUS: ***Chimerella*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolene mariaelenae* Cisneros-Heredia and McDiarmid, 2006a, and that is nested within Centroleninae.

TYPE SPECIES: *Centrolene mariaelenae* Cisneros-Heredia and McDiarmid, 2006a.

DIAGNOSIS: *Chimerella* is differentiated from other taxa by having: (1) adult males with small humeral spines, (2) lobed liver covered by a white hepatic

peritoneum, digestive tract white, (3) ventral parietal peritoneum completely transparent, (4) moderate webbing between Fingers III and IV, (5) pale green bones in life, (6) dorsum lavender in preservative, with small dark flecks, (7) dentigerous process of the vomer present, but edentate, (8) males call from the upper surfaces of leaves. *Chimerella mariaelenae* is morphologically similar to three species restricted to the Guianan Shield [*Vitreorana gorzulai* (Ayarzagüena 1992), *V. lema* (Duellman & Señaris 2003), and *V. papillahallica* (Noonan & Harvey 2000)]. However, *C. mariaelenae* is restricted to Andes.

CONTENT (1 SPECIES): *Chimerella mariaelenae* (Cisneros-Heredia and McDiarmid, 2006a) new combination.

ETYMOLOGY: The name *Chimerella* comes from the Greek *Chimaira*. In Greek mythology, the Chimera is a creature composed of parts of multiple animals. I use the name in reference to the peculiar combination of morphological characteristics present in *Chimerella mariaelenae.* The suffix *–ella* is a diminutive form, *Chimerella* is feminine in gender.

SISTER TAXON: The sister taxon of *Chimerella* is not clearly established (Figs. 2.7, 2.8). The mitochondrial tree supports the hypothesis that *C. mariaelenae* is sister to (*Vitreorana* new genus + *Teratohyla* Taylor, 1951 + *Rulyrana* new genus + *Sachatamia* new genus + *Cochranella* Taylor, 1951 + *Espadarana* new genus).

DISTRIBUTION: The only species in the genus, *Chimerella mariaelenae*, is known from three localities at elevations of 1400–1820 m on the Amazonian slopes of the Ecuadorian Andes (Cisneros-Heredia and McDiarmid, 2006a, Cisneros-

Heredia and Guayasamin, 2006; Fig. 2.14).

COMMENTS: Cisneros-Heredia and McDiarmid (2006a) argued that *Chimerella mariaelenae* is a part of a clade (the *gorzulai* species group, *sensu* Duellman and Señaris, 2003) endemic to the Guianan Shield. These authors presented their hypothesized clade as evidence supporting a biogeographical connection between the Andes and the Guianan Shield. Although the inclusion of *C. mariaelenae* in the *gorzulai* group is not supported by the molecular phylogeny, the relationships of these species are not resolved (Figs. 2.7, 2.8); therefore, additional data are necessary to support or reject the Andes-Guianan Shield hypothesis.

GENUS: ***Cochranella*** Taylor, 1951

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolenella granulosa* Taylor, 1949, *Centrolene litorale* Ruiz-Carranza and Lynch, 1996, and *Cochranella nola* Harvey, 1996.

TYPE SPECIES: *Centrolenella granulosa* Taylor, 1949.

DIAGNOSIS: I found no unambiguous, nonmolecular synapomorphies for the genus *Cochranella*. This clade is diagnosed by the following characters: (1) absence of humeral spines (small spine present in *C. litoralis*); (2) digestive tract white (translucent in *Cochranella nola*), lobed liver covered by a transparent hepatic peritoneum; (3) ventral parietal peritoneum white anteriorly and transparent posteriorly; (4) moderate to extensive webbing between Fingers III and IV; (5) bones

green in life; (6) dorsum lavender in preservative, with or without spots; (7) dentigerous process of the vomer and vomerine teeth present (absent in *C. litoralis*); (8) males call from the upper surfaces of leaves, and females deposit eggs on the upper sides of leaves along streams, (9) quadratojugal articulating with maxilla. *Cochranella* is differentiated from *Rulyrana* by usually having white visceral peritoneum (translucent in *Rulyrana*). Three species of *Centrolene* (*C. daidaleum,* C. *savagei,* and *C. solitaria*) are remarkably similar to some species in *Cochranella* [*C. mache* Guayasamin and Bonaccorso, 2004, *C. resplendens* (Lynch and Duellman, 1973)], and I am unaware of any discrete phenotypic trait that would unambiguously indicate their corresponding clade. However, there is an evident difference in the biogeographic area that they inhabit. All species of *Centrolene* are restricted to the tropical Andes, whereas the *Cochranella* mentioned are found only in lowlands (< 1000 m).

CONTENT (7 SPECIES): *Cochranella granulosa* (Taylor 1949), *C. euknemos* (Savage & Starrett 1967), *C. litoralis* (Ruiz-Carranza & Lynch 1996) new combination, *C. mache* Guayasamin & Bonaccorso 2004, and *C. nola* Harvey 1996. Additionally, based on morphology (see diagnosis), we consider *C. phryxa* Aguayo & Harvey 2006, and *C. resplendens* (Lynch & Duellman 1973) as part of the clade *Cochranella;* a hypothesis that needs to be tested with the use of molecular data.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). However, the topology inferred from the combined mitochondrial dataset supports a clade formed by *Cochranella* Taylor, 1951 + *Espadarana* new genus.

DISTRIBUTION: The genus *Cochranella* is distributed in the lowlands and mountains at elevations below 1750 m in Central America (*Cochranella granulosa, C. euknemos*), the Pacific lowlands and cloud forests of Colombia (*C. euknemos, C. litoralis*) and Ecuador (*C. litoralis, C. mache*), the Amazonian slopes of the Andes of Bolivia (*C. nola*), and the Amazonian lowlands of Ecuador (*C. resplendens*), Peru (*C. resplendens*), and Bolivia (*C. phryxa*). See Figure 2.15.

COMMENTS: Most of the species that are placed in *Cochranella* (*C. granulosa, C. euknemos, C. mache, C. resplendens*) used to be part of the *Cochranella granulosa* Group (*sensu* Ruiz-Carranza and Lynch, 1991a, b). The monophyly of *Cochranella* is strongly supported by 12S, 16S, combined mitochondrial, combined nuclear, and complete datasets.

GENUS: ***Rulyrana*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Cochranella adiazeta* Ruiz-Carranza and Lynch, 1991d, and *Centrolenella flavopunctata* Lynch and Duellman, 1973.

TYPE SPECIES: *Centrolenella flavopunctata* Lynch and Duellman, 1973.

DIAGNOSIS: The clade *Rulyrana* seems to lack unambiguous nonmolecular synapomorphies. The following characteristics are diagnostic: (1) humeral spines absent; (2) lobed liver covered by a transparent hepatic peritoneum, digestive tract translucent; (3) ventral parietal peritoneum white anteriorly and transparent posteriorly; (4) moderate to extensive webbing between Fingers III and IV; (5) bones

green in life; (6) dorsum lavender in preservative, with or without spots; (7) dentigerous process of the vomer present, vomerine teeth usually present [present or absent in *R. spiculata* (Duellman, 1976) and *R. flavopunctata*]; (8) males call from the upper surfaces of leaves, females deposit eggs on the upper sides of leaves. See comments.

CONTENT (6 SPECIES): *Rulyrana adiazeta* (Ruiz-Carranza and Lynch, 1991d) new combination, *R. flavopunctata* (Lynch and Duellman, 1973) new combination, *R. spiculata* (Duellman, 1976) new combination, *R. saxiscandens* (Duellman and Schulte, 1993) new combination, *R. susatamai* (Ruiz-Carranza and Lynch, 1995a) new combination, and *R. tangarana* (Duellman and Schulte, 1993) new combination. I place *R. saxiscandens* and *R. tangarana* in *Rulyrana* based on their morphology; these two species are nearly identical to *R. spiculata*.

ETYMOLOGY: *Rulyrana* is named in honor of Pedro Ruiz-Carranza and John D. Lynch, who have contributed enormously to the understanding of centrolenid diversity and evolution. The name *Rulyrana* comes from an arbitrary association of the two first letters of Ruiz and Lynch and the word *rana* (= frog). Additionally, *Ruly* happens to be the nickname of my friend and colleague Martín Bustamante; herein, I recognize his work on amphibian conservation. The name *Rulyrana* is feminine in gender.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). However, the topology inferred from the combined mitochondrial dataset supports a clade formed by *Rulyrana* new genus + *Teratohyla* Taylor, 1951.

DISTRIBUTION: The genus *Rulyrana* is distributed on the Amazonian slopes of the Andes (*R. flavopunctata, R. spiculata*) in Ecuador and Peru, the western slopes of the Cordillera Oriental of the Andes in Colombia (*R. adiazeta*), and the eastern slopes of the Cordillera Central of the Andes in Colombia (*R. susatamai*). See Figure 2.15.

COMMENTS: I am unable to find discrete phenotypic differences between *Rulyrana* and *Sachatamia* new genus. Ideally, these two taxa would have been placed in a single monophyletic genus, as suggested by the nuclear dataset (Fig. 2.8) and Bayesian analysis of the complete dataset (Fig. 2.7). However, the mitochondrial phylogeny indicates that *Rulyrana* and *Sachatamia* do not form a monophyletic group (Fig. 2.8). Given the incongruence among datasets, I prefer to recognize the two genera. If further work finds that *Rulyrana* + *Sachatamia* do form a natural group, it would be recommended to consider *Sachatamia* as a synonym of *Rulyrana*. At the moment, however, placement of species in either of these two genera requires the use of molecular data. The monophyly of the clade *Rulyrana* is well supported by 12S, RAG1, POMC, combined nuclear, combined mitochondrial, and complete datasets.

GENUS: ***Sachatamia*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolenella ilex* Savage, 1967, and *Centrolenella albomaculata* Taylor, 1949.

TYPE SPECIES: *Centrolenella albomaculata* Taylor, 1949.

DIAGNOSIS: Although the clade *Sachatamia* lacks unambiguous nonmolecular

synapomorphies, the following combination of characteristics is diagnostic: (1) humeral spines present (*S. ilex*) or absent [*S. albomaculata, S. punctulata* (Ruiz-Carranza and Lynch, 1995a)], (2) lobed liver covered by a transparent hepatic peritoneum, digestive tract translucent, (3) ventral parietal peritoneum white anteriorly and transparent posteriorly, (4) moderate to extensive webbing between Fingers III and IV, (5) bones green in life, (6) dorsum lavender in preservative, with or without spots, (7) dentigerous process of the vomer present, bearing teeth, (8) males call from the upper surfaces of leaves, females deposit pigmented eggs on the upper sides of leaves, (9) quadratojugal articulating with maxilla. Although phenotypic characters distinguish *Sachatamia* from most centrolenid taxa, there are no discrete differences between this genus and *Rulyrana;* therefore, DNA data are necessary to unambiguously allocate species in one of these two clades.

CONTENT (3 SPECIES): *Sachatamia albomaculata* (Taylor, 1949) new combination, *S. ilex* (Savage, 1967) new combination, *S. punctulata* (Ruiz-Carranza and Lynch, 1995a) new combination.

ETYMOLOGY: The name *Sachatamia* comes from the Quichua words *sacha,* meaning "forest," and *tamia,* meaning "rain," and refers to the tropical rainforest occupied by the clade. *Sachatamia* is feminine in gender.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). The mitochondrial topology supports a clade formed by *Sachatamia* new genus + (*Rulyrana* new genus + *Teratohyla* Taylor, 1951). The complete dataset suggests a *Sachatamia* + *Rulyrana* clade.

DISTRIBUTION: The genus *Sachatamia* is distributed in the rainforest at elevations below 1500 m in Central America (Honduras, Nicaragua, Costa Rica, Panama) and South America (Colombia, Ecuador). In South America, *Sachatamia ilex* and *S. albomaculata* occur in the Pacific lowlands, whereas *S. punctulata* is restricted to the eastern slopes of the Cordillera Central of the Colombian Andes. See Figure 2.15.

COMMENTS: The monophyly of *Sachatamia* is strongly supported by the 16S, ND1, combined mitochondrial, and complete datasets. See comments under *Rulyrana*.

GENUS: ***Espadarana*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolenella andina* Rivero, 1968, and *Hyla prosoblepon* Boettger, 1892.

TYPE SPECIES: *Centrolenella andina* Rivero, 1968.

DIAGNOSIS: *Espadarana* lacks any unambiguous, nonmolecular synapomorphies, however, the clade is diagnosed by the following characters: (1) adult males with conspicuous humeral spines, (2) lobed liver covered by a transparent hepatic peritoneum, digestive tract translucent, (3) ventral parietal peritoneum white anteriorly and transparent posteriorly, (4) moderate webbing between Fingers III and IV, (5) bones green in life, (6) dorsum lavender in preservative, with or without spots, (7) dentigerous process of the vomer bearing teeth, (8) males call from the upper

surfaces of leaves, and females deposit eggs on the upper sides of leaves over streams, (9) when fighting, males dangle by their feet and grapple venter-to-venter (Jacobson, 1985; Guayasamin and Barrio-Amorós, 2005; Fig. 2.3), (10) quadratojugal articulating with maxilla. The presence of conspicuous humeral spines in the adult males of species in *Centrolene* distinguishes this clade from most other centrolenids. The other two genera presenting this trait are *Centrolene* and the monotypic *Ikakogi* new genus. *Ikakogi tayrona* (Ruiz-Carranza and Lynch, 1991a) is the only centrolenid species where females guard egg clutches (Rada, unpublished data) and males have humeral spines and white bones in life. Most species in *Centrolene* lack teeth on the vomers (teeth present in *C. geckoideum* and *C. savagei*), whereas all species in *Espadarana* have vomerine teeth; however, DNA data are needed for the unambiguous placement of species in either of these two clades.

CONTENT (3 SPECIES): *Espadarana andina* (Rivero, 1968) new combination, *E. prosoblepon* (Boettger, 1892) new combination, *E. callistomma* (Guayasamin and Trueb, 2007) new combination.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). However, the topology inferred from the combined mitochondrial dataset supports a sister relationship between *Cochranella* Taylor, 1951 and *Espadarana* new genus.

ETYMOLOGY: The name *Espadarana* honors Marcos Jiménez de la Espada, a Spanish zoologist who was part of the Comisión Científica del Pacífico that explored America between 1862 and 1865. Jiménez de la Espada described the first centrolenid frog, *Centrolene geckoideum* in 1872. In Spanish, the word *Espada* means sword,

which I associate with the humeral spines present in the adult males of the species in this clade. *Espadarana* is a combination of the words *Espada* and *rana* (frog), and is feminine in gender.

DISTRIBUTION: Members of the genus *Espadarana* occur at elevations below 2500 m in the lowlands and mountains of Central America (*E. prosoblepon*), the Pacific lowlands of Colombia and Ecuador (*E. callistomma, E. prosoblepon*), and the cloud forests of the Andes in Colombia and Cordillera de Mérida in Venezuela (*S. andina*). See Figure 2.15.

COMMENTS: Adult males of *Centrolene, Espadarana,* and *Ikakogi* have pronounced humeral spines. Other species present small or hidden humeral spines (*Chimerella mariaelenae, Nymphargus grandisonae, Sachatamia ilex, Vitreorana lema, V. papillahallica, V. gorzulai*). The monophyly of *Espadarana* is strongly supported by 12S, 16S, ND1, POMC, combined mitochondrial, combined nuclear, and complete datasets.

GENUS: ***Teratohyla*** Taylor, 1951

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolenella spinosa* Taylor, 1949, and *Hyla pulverata* Peters, 1873.

TYPE SPECIES: *Centrolenella spinosa* Taylor, 1949, by original designation.

DIAGNOSIS: The most conspicuous characteristics of *Teratohyla* are: (1) humeral spines absent; (2) liver covered by a transparent [*T. midas* (Lynch and

Duellman, 1973), *T. spinosa*] or white [*T. pulverata, T. amelie* (Cisneros-Heredia and Meza-Ramos, 2007)] hepatic peritoneum, digestive tract translucent (*T. spinosa*) or white (*T. amelie, T. midas, T. pulverata*); (3) ventral parietal peritoneum white anteriorly and transparent posteriorly (*T. midas, T. spinosa*) or completely transparent (*T. amelie, T. pulverata*); (4) moderate to extensive webbing between Fingers III and IV; (5) bones pale to dark green in life; (6) dorsum creamy lavender to dark lavender in preservative, with or without spots; (7) dentigerous process of the vomer present, dentate (*T. pulverata*) or edentate (*T. amelie, T. midas, T. spinosa*); (8) males call from the upper surfaces of leaves, females deposit eggs on the tips of leaves [*T. pulverata,* Savage (2002)] or along the margins of the undersides of leaves [*T. spinosa,* Starrett (1960)]; (9) prepollical spine protruding (*T. spinosa*) or not protruding (*T. amelie, T. midas, T. pulverata*). Males of *T. midas* have a venter-to-venter fighting behavior (J. Bosh unpublished data).

CONTENT (4 SPECIES): *Teratohyla amelie* (Cisneros-Heredia and Meza-Ramos, 2007) new combination, *T. midas* (Lynch and Duellman, 1973) new combination, *T. pulverata* (Peters, 1873) new combination, and *T. spinosa* (Taylor, 1949) new combination.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). The results from the ML and Bayesian analysis of the mitochondrial dataset support a clade consisting of *Teratohyla* Taylor, 1951 + *Rulyrana* new genus.

DISTRIBUTION: *Teratohyla* occurs in the lowlands of Central America and in the Pacific and Amazonian lowlands of South America below 1000 m (Fig. 2.16).

COMMENTS: This clade is one of the few examples within Centrolenidae where the vicariant barrier hypothesized to cause speciation is the uplift of the Andes. According to Hoorn et al. (1995), the Eastern Cordillera in the Colombian Andes was a continuous range by late middle Miocene (12.10–11.8 mya). If this dating is correct, sister species in *Teratohyla* have been evolving independently for a long period of time. Despite this long isolation, the morphology of sister species has been maintained (see diagnosis above). Also, sister species still inhabit the lowlands (< 1000 m) of tropical rainforests on opposite sides of the Andes, suggesting that the niche has been conserved (see Peterson et al., 1999). The monophyly of *Teratohyla* is inferred with significant support from the combined nuclear and complete datasets.

GENUS: ***Vitreorana*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Centrolenella antisthenesi* Goin, 1963, and *Centrolenella gorzulae* Ayarzagüena, 1992.

TYPE SPECIES: *Centrolenella antisthenesi* Goin, 1963.

DIAGNOSIS: The genus *Vitreorana* is morphologically diverse and lacks unambiguous, nonmolecular synapomorphies. The most conspicuous feature of this clade is the presence of a white hepatic peritoneum covering the liver [or partially covering the liver in *V. oyampiensis* (Lescure, 1975)]; this trait is a synapomorphy at this level of the phylogeny. Also, most species in *Vitreorana* have a white gastrointestinal peritoneum [opaque in *V. eurygnatha* (Lutz, 1925)]. The combination

of these two traits distinguishes *Vitreorana* from most centrolenid clades, except *Hyalinobatrachium* and *Chimerella*. Species in *Hyalinobatrachium* deposit their eggs on the undersides of leaves, whereas most species in *Vitreorana* deposit their eggs on the upper surfaces of leaves, the only exception being *V. eurygnatha,* which has been observed to deposit its eggs on either the upper or the undersides of leaves (Lutz, 1947). Morphologically, *Chimerella mariaelenae* is similar to *V. gorzulai*, *V. lema,* and *V. papillahallica.* Although the phenotypic resemblance between these species suggest an evolutionary relatedness, none of the genes studied support a *Chimerella* + *Vitreorana* clade. See comments under *Chimerella.*

CONTENT (11 SPECIES): *Vitreorana ametarsia* (Flores, 1987) new combination, *V. antisthenesi* (Goin, 1963) new combination, *V. castroviejoi* (Ayarzagüena and Señaris, 1996) new combination, *V. eurygnatha* (Lutz, 1925) new combination, *V. gorzulai* (Ayarzagüena, 1992) new combination, *V. helenae* (Ayarzagüena, 1992) new combination, *V. lema* (Duellman and Señaris, 2003) new combination, *V. oyampiensis* (Lescure, 1975) new combination, *V. papillahallica* (Noonan and Harvey, 2000) new combination, *V. parvula* (Boulenger, 1895) new combination, *V. uranoscopa* (Müeller, 1924) new combination. See comments.

ETYMOLOGY: *Vitreorana* is derived from the Latin *vitreum,* meaning "glass," and the Latin *rana,* meaning "frog." The name refers to the total or partial transparency of the venter of these frogs. *Vitreorana* is feminine in gender.

SISTER TAXON: The sister clade of *Vitreorana* is not clearly established. The combined dataset suggests that *Vitreorana* is sister to a clade containing all the other

genera in Clade C, a result that has significant support when the mitochondrial dataset is analyzed under Bayesian criterion.

DISTRIBUTION: Members of *Vitreorana* occur at elevations below 1900 m in the Cordillera de la Costa of Venezuela (*V. antisthenesi, V. castroviejoi*), Guianan Shield (*V. gorzulai, V. helenae, V. lema, V. oyampiensis, V. papillahallica*), Amazonia of Colombia and Ecuador (*V. ametarsia*), and in the Atlantic Forest of Brazil and Argentina (*V. eurygnatha, V. parvula, V. uranoscopa*). See Figure 2.16.

COMMENTS: Based on morphology and behavior, I place *Hyalinobatrachium parvulum, H. uranoscopum,* and *Cochranella ametarsia* in the genus *Vitreorana*. *Vitreorana parvula* and *V. uranoscopa* are hypothesized to be close relatives of *V. eurygnatha,* for which the available molecular data strongly support placement in *Vitreorana.* Characters that suggest a close evolutionary relationship of the three species from the Atlantic Forest include the presence of guanophores on the urinary bladder, dentate vomers, green bones in life, and eggs usually deposited on the upper surface of leaves. The monophyly of the clade formed by *V. eurygnatha, V. parvula,* and *V. uranoscopa* already was suggested by Ruiz-Carranza and Lynch (1991a), who placed these taxa in the *Hyalinobatrachium parvulum* species group. *Cochranella ametarsia* is almost identical to *V. oyampiensis,* and I assume that this similarity is based on common ancestry. The monophyly of *Vitreorana* is strongly supported by 16S, the combined mitochondrial, combined nuclear, and complete datasets.

SUBFAMILY: **Hyalinobatrachinae** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Hylella fleischmanni* Boettger, 1893, and *Centrolenella revocata* Rivero, 1985.

TYPE GENUS: *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991a.

DIAGNOSIS: Two derived behavioral character states are present in most species of Hyalinobatrachinae. Males in all species in *Hyalinobatrachium* [except *H. taylori* (Goin, 1968), see Ayarzagüena, 1992; Señaris and Ayarzagüena, 2005] and at least one of the two species of *Celsiella* (*C. revocata*) call from the undersides of leaves (Ruiz-Carranza and Lynch, 1998; Señaris and Ayarzagüena, 2005). Females of *Hyalinobatrachium* deposit their egg masses on the undersides of leaves (Ruiz-Carranza and Lynch, 1998), *Celsiella revocata* deposits eggs on both the uppersides and undersides of leaves, whereas *C. vozmedianoi* (Ayarzagüena and Señaris, 1996) deposit the eggs on the uppersides of leaves (Señaris and Ayarzagüena, 2005; Castroviejo-Fisher pers. obs.). Additional synapomorphies of Hyalinobatrachinae are the presence of a reduced prepollex (relative length < 50% of Metacarpal I), and the complete fusion between the tibiale and fibulare (Figs. 2.1, 2.12).

CONTENT (2 GENERA): *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991a, as modified in this work, and *Celsiella* new genus.

SISTER TAXON: Uncertain (Figs. 2.7, 2.8). At the base, the family Centrolenidae has a trichotomy; therefore, further gene sampling is needed to resolve the relationships among Centroleninae, Hyalinobatrachinae, and *Ikakogi tayrona*.

DISTRIBUTION: Representatives of Hyalinobatrachinae occur at elevations up to 2500 m in tropical Central America, the tropical Andes, the coastal mountains of Venezuela, the upper Amazon Basin, and the Guianan Shield (Fig. 2.17).

COMMENTS: The monophyly of the subfamily Hyalinobatrachinae is strongly supported by 12S, 16S, combined mitochondrial, combined nuclear, and complete datasets.

GENUS: ***Hyalinobatrachium*** Ruiz-Carranza and Lynch, 1991a

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Hylella fleischmanni* Boettger, 1893, and *Centrolenella taylori* Goin, 1968.

TYPE SPECIES: *Hylella fleischmanni* Boettger, 1893, by original designation.

DIAGNOSIS: The genus *Hyalinobatrachium*, as defined by Ruiz-Carranza and Lynch (1991a), is polyphyletic. Herein, I restrict the name *Hyalinobatrachium* to the species that Savage (1967) and Ruiz-Carranza and Lynch (1991a) referred to as the *fleischmanni* Group. The following character states unambiguously diagnose *Hyalinobatrachium:* (1) humeral spines absent (Savage, 1967); (2) liver and digestive tract covered by white peritonea (Savage, 1967); (3) completely transparent ventral parietal peritoneum (Savage, 1967; Fig. 2.1); (4) white bones in life (Savage, 1967); (5) dorsal coloration in preservative white or cream (Savage, 1967); (6) males lack conspicuous dorsal spinules during breeding season; (7) nuptial pad small and restricted to the inner edge of Finger II in males (Type V of Cisneros-Heredia and

McDiarmid, 2007; Fig. 2.4); (8) dentigerous process of the vomer and vomerine teeth absent (Ruiz-Carranza and Lynch, 1991a; Savage, 1967); (9) males usually vocalize from the undersides of leaves, and females deposit one layer of eggs on the undersides of leaves (Ruiz-Carranza and Lynch, 1998); (10) when fighting, males assume an amplexus-like position (Bolívar et al., 1999) in the few taxa that have been observed (Guayasamin and Barrio-Amorós, 2005; Kubricki, 2007); and (11) complete fusion of tibiale and fibulare. Other potential synapomorphies of *Hyalinobatrachium* are the possession of small nasal bones widely separated from one another (Barrera-Rodríguez, 2000; Señaris and Ayarzagüena, 2005; Cisneros-Heredia and McDiarmid, 2006a; this work), a reduced prepollex, and two exposed parietal fontanelles (Señaris and Ayarzagüena, 2005; for definition see Guayasamin and Trueb, 2007). Exposed parietal fontanelles are evident in *H. colymbiphyllum* (Taylor, 1949), *H. crurifasciatum* Myers and Donnelly, 1997, *H. duranti* (Rivero, 1985), *H. esmeralda* Ruiz-Carranza and Lynch, 1998, *H. fleischmanni, H. fragile* (Rivero, 1985), *H. iaspidiense* (Ayarzagüena, 1992), *H. mondolfii* Señaris and Ayarzagüena, 2001, *H. orientale* (Rivero, 1968), *H. talamancae* (Taylor, 1952), and *H. taylori* (Señaris and Ayarzagüena, 2005, this study); however, this character state is not unambiguous for *Hyalinobatrachium* because the parietal fontanelles are partially or completely covered by bone in *H. aureoguttatum* (Barrera-Rodríguez and Ruiz-Carranza, 1989), *H. bergeri* (Cannatella, 1980), and *H. chirripoi* (Taylor, 1958). Additionally, *Espadarana andina, Centrolene hesperium, Vitreorana eurygnatha,* and *V. uranoscopa* present a similar derived character state (i.e., exposed parietal

fontanelles).

CONTENT (29 SPECIES): *Hyalinobatrachium aureoguttatum* (Barrera-Rodríguez & Ruiz-Carranza 1989), *H. bergeri* (Cannatella 1980), *H. chirripoi* (Taylor 1958), *H. colymbiphyllum* (Taylor 1949), *H. crurifasciatum* Myers & Donnelly 1997, *H. duranti* (Rivero 1985), *H. eccentricum* Myers & Donnelly 2001, *H. esmeralda* Ruiz-Carranza & Lynch 1998, *H. fleischmanni* (Boettger 1893), *H. fragile* (Rivero 1985), *H. guairarepanense* Señaris 2001, *H. iaspidiense* (Ayarzagüena 1992), *H. ibama* Ruiz-Carranza & Lynch 1998, *H. ignioculus* Noonan & Bonett 2003, *H. lemur* Duellman & Schulte 1993, *H. mondolfii* Señaris & Ayarzagüena 2001, *H. munozorum* (Lynch & Duellman 1973), *H. nouraguense* Lescure & Marty 2000, *H. orientale* (Rivero 1968), *H. orocostale* (Rivero 1968), *H. pallidum* (Rivero 1985), *H. pellucidum* (Lynch & Duellman 1973), *H. petersi* (Goin 1961), *H. ruedai* Ruiz-Carranza & Lynch 1998, *H. talamancae* (Taylor 1952), *H. tatayoi* Castroviejo-Fisher, Ayarzagüena, & Vilà 2007, *H. taylori* (Goin 1968), *H. valerioi* (Dunn 1931), *H. vireovittatum* (Starrett & Savege 1973). Although placement of most species in the genus *Hyalinobatrachium* was based on molecular data, a few were allied only on the basis of morphological and behavioral characteristics (Appendix 2.2); given that *Hyalinobatrachium* is a well-defined clade (see diagnosis), the assignations of these species into the genus is unambiguous.

SISTER TAXON: *Celsiella* new genus. The monophyly of the clade *Hyalinobatrachium* + *Celsiella* is supported by the topologies inferred from the 12S, 16S, combined nuclear, combined mitochondrial, and complete datasets.

DISTRIBUTION: *Hyalinobatrachium* has a wide distribution that includes tropical Central America, the tropical Andes, the Cordillera de la Costa of Venezuela, the upper Amazon Basin, and the Guianan Shield, at elevations between sea level and 2500 m (Fig. 2.17).

COMMENTS: Ruiz-Carranza and Lynch (1991a, 1998) described *Hyalinobatrachium* and recognized three species groups within the genus (i.e., *fleischmanni*, *pulveratum*, and *parvulum*). In Chapter 1, I showed that *Hyalinobatrachium* (sensu Ruiz-Carranza and Lynch, 1991a) is polyphyletic. However, the *H. fleischmanni* Group (sensu Ruiz-Carranza and Lynch, 1998) is a monophyletic clade and I restrict the name *Hyalinobatrachium* to it. As noted above, *Hyalinobatrachium* (sensu stricto) is distinct morphologically and behaviorally. However, several of the traits mentioned in the diagnosis occur in other clades. For example, many species in *Vitreorana* have a transparent ventral parietal peritoneum and a white liver. Other species (*Celsiella revocata*, *Centrolene peristictum*) also are known to call and deposit eggs on the undersides of leaves (Señaris and Ayarzagüena, 2005, M. R. Bustamante, pers. comm.). Other characters, such as the absence of humeral spines, dentigerous process of the vomer, and the complete fusion of the tibiale and fibulare are widespread in the family. *Hyalinobatrachium,* as defined in this work, is strongly supported by the combined mitochondrial and the complete datasets.

GENUS: ***Celsiella*** new clade name

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon stemming from the most recent common ancestor of *Cochranella vozmedianoi* Ayarzagüena and Señaris, 1996, and *Centrolenella revocata* Rivero, 1985.

TYPE SPECIES: *Centrolenella revocata* Rivero, 1985.

DIAGNOSIS: Although *Celsiella* lacks unambiguous, nonmolecular synapomorphies, the two species in the genus can be differentiated from most other centrolenid genera by the combination of the following characters: (1) humeral spines absent; (2) trilobed liver, covered by a clear hepatic peritoneum; (3) ventral parietal peritoneum white anteriorly and transparent posteriorly; (4) bones pale green or green in life; (5) dorsum mainly cream in preservative; (6) males lack conspicuous spinules on the dorsum; (7) nuptial pads inconspicuous; (8) vomer lacking dentigerous process and teeth; (9) males call from the upper side of leaves in *C. vozmedianoi,* and from the upper- or undersides of leaves in *C. revocata* (Señaris and Ayarzagüena, 2005), females deposit eggs on the uppersides of leaves along streams in *C. vozmedianoi,* and on the upper- or undersides of leaves in *C. revocata* (Señaris and Ayarzagüena, 2005); (10) tibiale and fibulare fused. The genera *Nymphargus* and *Rulyrana* are morphologically similar to *Celsiella.* None of the species in *Nymphargus* and *Rulyrana* is known to deposit eggs on the underside of leaves. Furthermore, *Celsiella* is restricted to the Cordillera de la Costa of Venezuela, whereas *Nymphargus* and *Rulyrana* are endemic to the Andes.

CONTENT (2 SPECIES): *Celsiella vozmedianoi* (Ayarzagüena and Señaris, 1996) new combination, and *C. revocata* (Rivero, 1985) new combination.

ETYMOLOGY: I am pleased to name this taxon after Josefa Celsa Señaris "Celsi" in recognition of her contributions to the knowledge of centrolenid diversity and morphology. The suffix *–ella* is a diminutive form, and *Celsiella* is feminine in gender.

SISTER TAXON: *Hyalinobatrachium* Ruiz-Carranza and Lynch, 1991a, as modified in this work.

DISTRIBUTION: *Celsiella* is endemic to the Cordillera de la Costa of Venezuela. *Celsiella revocata* is found in the Cordillera de la Costa at elevations between 1200 and 1800 m, *C. vozmedianoi* has been reported only from Cerro Humo, Península de Paria at 750–780 m (Señaris and Ayarzagüena, 2005).

COMMENTS: The clade *Celsiella* is strongly supported by the 12S, 16S, ND1, *c-myc*, combined mitochondrial, combined nuclear, and complete datasets.

INCERTAE SEDIS WITHIN CENTROLENIDAE: ***Ikakogi*** new genus

PHYLOGENETIC CHARACTERIZATION: A monophyletic taxon nested within Centrolenidae that includes *Centrolene tayrona* Ruiz-Carranza and Lynch, 1991b.

TYPE SPECIES: *Centrolene tayrona* Ruiz-Carranza and Lynch, 1991b.

DIAGNOSIS: The combination of the following characteristics differentiates this taxon from other genera: humeral spines in adult males, white bones in life, ventral parietal peritoneum white anteriorly and transparent posteriorly, and hepatic and visceral peritonea transparent. There are two unusual behaviors in *Ikakogi tayrona.* So far, it is the only known centrolenid species in which females guard eggs

clutches (Rada, unpublished data). All other species studied show paternal care (Ruiz-Carranza and Lynch, 1998; Señaris and Ayarzagüena, 2005; Rada, unpublished data). Additionally, *I. tayrona* is polymorphic for the egg deposition site, egg clutches have been found on the upper and lower surfaces of leaves (Rada, unpublished data).

CONTENT (1 SPECIES): *Ikakogi tayrona* (Ruiz-Carranza and Lynch, 1991b).

ETYMOLOGY: The name *Ikakogi* makes reference to the Ika (or Ijka) and Kogi people, descendants of the Tayrona, who inhabit the Sierra Nevada de Santa Marta.

DISTRIBUTION: *Ikakogi tayrona* inhabits the cloud forests of the Sierra Nevada de Santa Marta (Ruiz-Carranza and Lynch, 1991b), a mountain range completely isolated from other Andean moist forests by lower-elevation dry forests and xeric shrub lands.

COMMENTS: The phylogenetic position of *Ikakogi tayrona* (Figs. 2.7, 2.8) indicates that this species diverged early in the evolutionary history of Glassfrogs. Although its external morphology is similar to that of species in *Centrolene,* the behavior of *I. tayrona* is certainly unusual, as mentioned above. Two nuclear genes (POMC, RAG1) show a weak support for a *Nymphargus* + *Ikakogi* clade; however, all the other genes were unable to resolve the placement of *I. tayrona*. Data from more molecular loci are necessary to establish the phylogenetic position of *I. tayrona;* until then, I consider this species as *incertae sedis* within Centrolenidae.

DISCUSSION

Centrolenidae, Allophrynidae, and Neobatrachia

The phylogenetic position of Centrolenidae within the diversity of

Neobatrachia has been debated but not resolved with confidence either by molecular studies (Austin et al., 2002; Biju and Bossuyt, 2003; Darst and Cannatella, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006; Roelants et al., 2007) or by phenotypic data (Jiménez de la Espada, 1872; Noble, 1931; Taylor, 1951; Lynch, 1974; Duellman and Trueb, 1994; Ford and Cannatella, 1993; Rueda-Almoacid, 1994; Duellman, 2001; Haas, 2003; Burton, 2004; Wiens et al., 2005). I attempted to address this issue in Chapter 1 using molecular data, and obtained results supporting an Allophrynidae + Centrolenidae Clade. I propose the Subsuperfamily Centrolenia for the Centrolenidae + Allophrynidae Clade, which was considered a family (i.e., Centrolenidae) by Frost et al. (2006). This arrangement has the virtue of maintaining the names that have been used in recent decades (i.e., Centrolenidae, Allophrynidae), but the drawback of applying an uncommon rank (Subsuperfamily).

When naming clades under the rules of the ICZN (1999), taxonomists should promote name stability when possible, even if that means applying ranks that are not commonly used. In a ranked system such as the ICZN, there are multiple available ranks above the genus and below the superfamily (Article 35.1; ICZN); therefore, changing established ranks to accommodate new hypotheses of relationships is not always necessary.

The relationships of Centrolenia and other anurans remain uncertain. Although some studies suggest Leptodactylidae or Leiuperidae as the sister taxon of Centrolenia (Frost et al., 2006; Chapter 1), it is evident that more nuclear loci will be

necessary to clarify the relationships within Hyloidea.

Advantages and drawbacks of the new taxonomy

There is consensus among biologists that taxonomic classifications should reflect evolutionary history and relationships between organisms. However, it is difficult to imagine that any classification system or phylogenetic methodology will ever be "perfect" because of the complexity of the processes involved in the evolution of life (Dubois, 2006). Some biologists (e.g., Mayr and Bock, 2002) have expressed the limitations of a cladistic approach to study, represent, and classify the diversity of life within the theoretical framework of evolutionary biology. The main difficulties are that taxa are not always monophyletic because of different speciation processes (i.e., anagenic and reticulated evolution; e.g., Grant 1981; Funk and Omland, 2003), and that rather than signaling low resolution in the phylogenetic hypothesis, polytomies may represent real patterns of speciation (McCracken and Sorenson, 2005). The dichotomous branching pattern of cladistics is, like any model in science, a simplification of reality. However, cladistics provides a testable and objective hypothesis for classifications. This proposal is based on a large dataset including different genes, and I have ranked clades clearly and congruently monophyletic; thus, I expect that phenomena such as those described above are not affecting these particular clades.

One of the disadvantages of the classification presented herein is the number of new genera described. Since Jiménez de la Espada described the first Glassfrog in

1872, six genera have been recognized (*Centrolene, Centrolenella, Cochranella, Hyalinobatrachium, Nymphargus,* and *Teratohyla*), and the new taxonomy adds seven new genera. Nevertheless, the recognition of these new genera is a necessary step to establish a phylogenetic taxonomy.

Last, the phylogenetic position of the new genus *Ikakogi* remains uncertain*;* hence, I treated it as *incerta sedis.* There is agreement between the mitochondrial and nuclear datasets in suggesting that *I. tayrona* is an early divergent species within Centrolenidae. Given that the Sierra Nevada de Santa Marta is an older geological formation than the Andes (Tschanz et al., 1974), it is not surprising that old lineages are found in the area. Additionally, the isolation of the Sierra Nevada from other cloud forests offers an explanation for the occurrence of only one species in the clade (i.e., lack of possibilities for dispersal and speciation).

To summarize, I have attempted to provide a scheme whereby genera are presented as natural groups that have statistical support and phenotypic diagnosability. The new taxonomic proposal represents an improvement toward understanding, communicating, and interpreting the evolution of Glassfrogs.

Generic placement of species

The mechanics of taxonomy require the use of the binomen (genus and species). Therefore, users of this (or any) taxonomy are accepting, explicitly or implicitly, a phylogenetic hypothesis of relationships given by the genus. I could not assign with enough confidence around 17% of the described species of Glassfrogs

(Appendix 2.2) because molecular data was not available and phenotypic traits were ambigous. We imagine that other taxonomists will encounter similar difficulties when describing new species based solely on morphological characters. However, investigators always can tentatively assign species to a genus, and their hypothesis will be re-evaluated as more data become available. Alternately, sequencing short fragments of DNA would allow assigning species to clades with high confidence (Wiens et al. 2005). Cases in which we are uncertain about the generic placement of a particular species are listed in Appendix 2.2. Individuals working with centrolenid frogs are invited to follow the system presented in Appendix 2.2, in which phylogenetic uncertainty is clearly labeled.

Unresolved Clades

Throughout the Results section, I have emphasized that the relationships among some clades are unresolved. Polytomies and poorly supported clades can result from several evolutionary processes, combined with the quality of the data and the way in which it is analyzed, and deviations between the gene and species phylogenies. For example, rapid ancient radiations will produce hard polytomies, whereas successive branching of lineages with relatively short and poorly supported internode lengths produces soft polytomies. In other cases, poor resolution is a function of the quality of the data, which may prove to not be variable enough at the systematic level, or simply, shortcomings in the analyses and the ways in which they are applied. Finally, gene phylogeny can differ from the species phylogeny as a result

of undetected gene duplication (i.e., hidden paralogy), lineage sorting of multiple alleles, and horizontal gene transfer or gene conversion.

There is accumulating evidence indicating that the radiation of Hyloidea corresponds to a rapid and ancient event (e.g., Darst and Cannatella, 2004; Wiens et al., 2005; Roelants et al., 2007, Chapter 1). I speculate that this could also be the case in some clades of Centrolenidae that likely radiated in parallel with the uplift of the Andes.

In principle, the relationships underlying soft polytomies can be resolved by adding data. However, the type and amount of data necessary to solve these problems are uncertain, and currently the subject of an intense debate among phylogeneticists (e.g., Whitfield and Lockhart, 2007; Philippe and Telford, 2006; Gatesy et al., 2007). Expressed sequence tags, genome-level characters, large genome comparisons, and sequence of more than 20 genes have been proposed as ways to improve phylogenetic accuracy (e.g., Rokas et al., 2003; Boore, 2006; Philippe and Telford, 2006; Whitfield and Lockhart, 2007; Gatesy et al., 2007).

The gap between gene trees and species trees seems to be a recurrent problem when inferring phylogenies from molecular data. Phylogenetic networks can help to visualize reticulated patterns such as those arising from hybridization and conflict between data that produce an impoverishment of the phylogenetic tree-like cladistic schema. Several methods have been developed for this purpose (reviewed in Whitfield and Lockhart, 2007). Also, a Bayesian approach recently was developed to estimate species trees from gene trees (Liu and Pearl, 2007; Edwards et al., 2007), a

venue that should be explored in the future.

Conclusions

I formalize the evolutionary proximity of Centrolenidae and Allophynidae with the name Centrolenia. This arrangement maintains the validity and species content of the two families included in Centrolenia, avoiding nomenclatural instability (Fig. 2.5). For an alternate taxonomy, see Frost et al. (2006).

The proposed taxonomy of Glassfrogs (Fig. 2.7) is an attempt to formalize the recent findings presented in a previous study (Chapter 1). The new taxonomy drastically differs from previous arrangements, which, in most of the cases recognized polyphyletic groups. A limitation of the proposal is that is based on an incomplete taxon sampling (55% of the recognized Glassfrogs). Although diagnoses based on phenotypic traits are provided, there are several cases in which the allocation of species is ambiguous when molecular data are lacking (Appendix 2.2); I encourage researchers interested in the centrolenids frogs to focus their efforts on these species. Another unanticipated result that deserves further investigation is the evolutionary relationship of *Ikakogi tayrona* and other Glassfrogs. The data at hand suggest that *I. tayrona* is the only surviving lineage of a clade that is as old as the subfamilies Hyalinobatrachinae and Centroleninae. If this hypothesis is confirmed, studying the morphology and behavior of *I. tayrona* would be of great importance for understanding the evolution of traits and biogeography of Glassfrog.

CHAPTER 3

EVOLUTION OF TRANSPARENCY, BEHAVIOR, AND SEXUALLY DIMORPHIC TRAITS IN GLASSFROGS (CENTROLENIDAE)

Traditionally, the repeated evolution of complex traits has been considered unlikely because it implies multiple occurrences of the same complex evolutionary trajectory (i.e., identical selective pressures, gene interactions, and adaptations). Under this assumption, morphological and/or behavioral traits that are unique in a group commonly are considered as key indicators of phylogenetic relationships. Now, these hypotheses are being revisited with the tools of molecular systematics. In light of this new phylogenetic framework, several empirical studies suggest that complex morphological and life-history traits have reappeared after having been lost in an evolutionary lineage (Omland and Lanyon, 2000; Collin and Cipriano, 2003; Santos et al., 2003; Whiting et al., 2003; Chippindale et al., 2004; Mueller et al., 2004; Kohlsdorf and Wagner, 2006; Wiens et al., 2007).

Herein, I focus on the evolution of Glassfrogs (Centrolenidae), a diverse, monophyletic, Neotropical group that currently contains 147 known species (AmphibiaWeb, 2007). The monophyly of centrolenids is well supported by morphological (Taylor, 1951; Hayes and Starrett, 1980; Ruiz-Carranza and Lynch, 1991a; Burton, 1998, 2004; Wiens et al., 2005; Guayasamin et al., 2006) and molecular data (Darst and Cannatella, 2004; Faivovich et al., 2005; Wiens et al., 2005; Frost et al., 2006; Grant et al., 2006); a recent phylogeny (Chapter 1) provided

the necessary framework for the study of character evolution. Glassfrogs possess physical and behavioral features that are rare among anurans; in this study, I concentrate on these traits—ventral transparency (Fig. 3.1), presence/absence of a humeral spine (Fig. 3.2), oviposition site of eggs (Fig. 3.1), and presence/absence of iridophores on the hepatic, gastrointestinal, and pericardial peritonea (Fig. 3.1).

The widespread ventral transparency in Glassfrogs is puzzling. Johnsen (2001) suggested that terrestrial organisms usually do not use transparency as a camouflage mechanism because of the low refractive index of air, and the effects of gravity and high levels of ultraviolet radiation. Moreover, it seems unlikely that ventral transparency is associated with concealment in Glassfrogs, because only the dorsum of these anurans is exposed to potential predators, and previous studies have shown that the dorsal surface provides camouflage by having the same reflective properties as photosynthetic leaves (Schwalm et al., 1977). The venters of all Glassfrogs are partially or completely transparent (Fig. 3.1A); therefore, it is reasonable to assume that this feature appeared in the ancestor of the clade. However, I have no way of assessing its adaptive value, if any, for lack of a hypothesis to test. Whereas benefits of transparency are equivocal, a drawback is patently obvious—viz., transparency of the ventral parietal peritoneum exposes internal organs to deleterious ultraviolet light. Based on the observation that species that are completely transparent ventrally usually have white iridophores in the peritoneum surrounding internal organs (i.e., liver and digestive tract), suggests that transparency evolved in tandem with the evolution of protective lining. I assess the significance of this

hypothesized correlation below.

Other unique morphological and behavioral characters that have evolved in Glassfrogs include the presence of humeral spines in adult males, a visible red heart, and deposition of eggs on the undersides of leaves (Figs. 3.1, 3.2). Based on the assumption that unique, complex traits provide accurate phylogenetic signals, the aforementioned characters have been considered as homologous and evolutionarily unambiguous, leading to taxonomic arrangements that were widely accepted (Ruiz-Carranza and Lynch, 1991a, 1998). I test the validity of these putative synapomorphies here.

Last, I assess the influence of incomplete taxon sampling in the reconstruction of ancestral character states. Evolutionary biologists have expressed concern as to the reliability of conclusions drawn from studies on character evolution in which taxon sampling is conspicuously incomplete (Cunningham, 1999). Simulations show that randomly increasing taxon sampling results in increased accuracy of character reconstruction (Salisbury and Kim, 2001) and phylogenetic inference (Zwickl and Hillis, 2002). Herein, I adopt a pragmatic approach and compare the topologies and character evolution inferred from an original dataset of about 100 taxa and a pruned dataset from which 50% of the species were randomly excluded. Also, I assess the influence of removing species with uncertain phylogenetic positions, one of which seems to have diverged early in centrolenid history.

I find that all traits previously hypothesized as having a unique origin have evolved and/or been lost multiple times, and that the evolution of complete

transparency is significantly correlated with the presence of protective iridophores on liver and digestive tract. The inferred history of humeral spines is ambiguous and could be dominated by multiple gains and few losses or one gain and several losses, depending on the methods used for the reconstruction. Finally, given the available taxon and gene sampling (~100 taxa, 4362 bp), the topology and interpretation of character evolution remain reasonably stable even when 50% of the species are excluded. However, when certain early divergent species are excluded, the history reconstructed for some traits changes changes conspicuously.

MATERIALS AND METHODS

Phylogenetic inference

As a phylogenetic framework, I used a molecular phylogeny inferred in Chapter 1. Taxon sampling was broad and included about 55% of the described species in Centrolenidae, representing all known morphological and behavioral variation of the group. For phylogenetic inference, I used the program RAxML (Randomized Axelerated Maximum Likelihood for High Performance Computing 2.2.0; Stamatakis, 2006; available at <http://icwww.epfl.ch/~stamatak/index-Dateien/Page443.htm>), which uses a GTR + CAT (GTR with per-site rate categories) approximation as replacement for GTR + Γ, and allows the use of mixed models of nucleotide substitution (Stamatakis, 2006); taking advantage of this feature, I partitioned the combined dataset by gene, and by codon position in protein coding genes. The resulting topology is identical to the one presented in Chapter 1. Nodal

support was estimated from Parsimony, ML, and Bayesian criteria as described in Chapter 1. Species names of centrolenid frogs follow the taxonomy proposed in Chapter 2.

Hypothesis Testing

Probabilistic approaches to testing hypotheses include parametric (Hulsenbeck and Bull, 1996; Hulsenbeck et al., 1996; Swofford et al., 1996; Goldman et al., 2000) and nonparametric (Shimodaira and Hasegawa, 1999) ML test, and Bayesian inference (Rannala and Yang, 1996; Yang and Rannala, 1997; Larget and Simon, 1999; Mau et al., 1999; Li et al., 2000; Huelsenbeck and Ronquist, 2001). Given that parametric tests are susceptible to misspecification of evolutionary model, and thus, prone to Type-I error (Huelsenbeck et al., 1996; Buckley, 2002), I prefer the Shimodaira-Hasegawa test (SH; Shimodaira and Hasegawa, 1999), which is more conservative, even under high substitution rates and branch-length heterogeneity (Buckley, 2002). I compared the topology of the "best tree" with that of prior hypotheses (Ruiz-Carranza and Lynch, 1991a, 1998) showing single origins for each of the following traits: complete ventral transparency; humeral spine; white liver; eggs deposited on the underside of leaves; and red heart visible. Theoretically, the SH test requires that all possible topologies are compared simultaneously, an obvious impossibility when numerous taxa are included. Hence, the number of candidate topologies was minimized by application of prior knowledge (Buckley, 2002). I searched for the best tree compatible with each prior hypothesis using the complete

dataset partitioned by gene and codon position and the program RAxML (Stamatakis, 2006).

Character evolution and the effect of taxon sampling on ancestral reconstruction

Morphological and behavioral characters are described in Appendix 3.1. I coded the character states through direct observation of specimens or by relying in the literature (Appendix 3.2). The most relevant sources for information on behavior came from Ruiz-Carranza and Lynch (1998) and Señaris and Ayarzagüena (2005). When coding morphological characters, multiple individuals of the same species were examined to assess the presence of polymorphisms. I limited my observation to adult males and adult females to avoid possible ontogenetic changes in character states.

Reconstruction of ancestral states was conducted using parsimony and maximum likelihood (ML) criteria in Mesquite 1.12 (Maddison and Maddison, 2006). Parsimony reconstructs the ancestral character states that minimize the number of changes required to produce the observed diversity, whereas ML solutions reconstruct the ancestral states that maximize the probability of the data given a model of evolution (Maddison et al., 1984; Pagel, 1999; Lewis, 2001). In likelihood reconstructions, the best estimate of the character state at each node was determined using the likelihood ratio test. When the log likelihoods of two states differed by 2.0 or more units, the state with better likelihood was considered the best estimate for that branch with strong statistical support (Pagel, 1999). When the difference in log likelihoods was < 2.0, the reconstruction was considered ambiguous. I used a model

with a single rate for gains and losses (1-parameter Markov k-state model; Mk1; Lewis, 2001), and also a model that allows for different rates of gain and loss (Asymmetrical 2-parameter Markov k-state mode) as implemented in Mesquite 1.12 (Maddison and Maddison, 2006). To test if the two-parameter model had a significantly better fit to the data than the one-parameter model, I used a likelihood ratio test. Because the two models are nested, I assessed the significance using a chi-square test with one degree of freedom (Mooers and Schluter, 1999; Pagel, 1999). For a discussion of limitations of parsimony and likelihood ancestral-character reconstruction see Pagel (1999), Cunningham (1999), and Schluter et al. (1997). Although Bayesian methods have been developed to account for phylogenetic uncertainty when estimating ancestral characters (Huelsenbeck et al., 2003; Pagel et al., 2004), I preferred to focus on the way in which taxon sampling affects rates of character gain and loss without including the effect of nodal support (discussed below).

Given that the phylogeny only includes 55% of the described diversity of Glassfrogs, I attempted to replicate the possible effect of incomplete sampling on the reconstruction of character evolution. For this, I randomly removed 50% of the species of the ingroup (Appendix 3.3) and inferred the phylogeny using RAxML and reconstruct the ancestral character states as described above. In a second approach, I removed two species (*Ikeakogi tayrona* and *Chimerella mariaelenae*) that have unstables position in the tree, as well as unusual combinations of morphological traits; then, again, I estimated the phylogeny and ancestral states. The resulting

reconstructions of the two strategies were compared with those performed under the tree with original sampling. To compare the topological differences between the trees, the symmetric distance measure (Robinson and Foulds, 1981) was calculated using the program Topological Distance (Puigbò et al., 2007) and normalized as described in Rosenberg and Kumar (2001). To make this comparison adequate, the tree from complete dataset was reduced to contain the same species as the pruned tree. The resulting value (phylogenetic error per internal branch = *E*) ranges from 0 to 1. Trees that are identical have $E = 0$, whereas completely different topologies have an $E = 1$.

Character correlation

To assess statistical correlation between two traits, I used the Concentrated Changes Test (CCT; Maddison, 1990) as implemented in MacClade 4.07 (Maddison and Maddison, 2005). The CCT evaluates whether origins of one specified character state are more concentrated than expected by chance on branches with another specified character state. A total of 100,000 datasets was simulated to create a random distribution representing the null hypothesis (i.e., the two characters evolving independently), with the ancestral state unspecified and actual changes. I tested the following correlations: complete ventral transparency vs. white digestive tract; and complete ventral transparency vs. white hepatic peritoneum. Because the CCT is sensitive to the inclusion of taxa (Maddison, 1990; Sillén-Tullberg, 1993; Lorch and Eadie, 1999), outgroups where excluded from these analyses.

RESULTS

Phylogeny and hypothesis testing

As already mentioned in Chapter 1, the phylogeny of Glassfrogs as inferred from molecular loci (Fig. 3.3) is highly incongruent with previous classifications of the group, which were based on the assumption that morphological and behavioral traits have evolved only once in centrolenids (Ruiz-Carranza and Lynch, 1991a, 1998). The SH test rejects ($P < 0.001$) constrained trees hypothesizing a single origin of complete ventral transparency, humeral spines, white liver, eggs deposited on the underside of leaves, and red heart visible, in favor of the alternate topology (Figs. 3.4, 3.5) that implies a more complex pattern of evolution (described below).

Although most of the nodes have a good support (Fig. 3.4), there is uncertainty in the phylogenetic position of *Ikeakogi tayrona* and *Chimerella mariaelenae*. This uncertainty is not the product of incomplete gene sampling (all genes were sequenced for the two taxa). However, it is possible that these species represent old lineages and that more genes will be needed to resolved their placement with confidence.

Effect of taxon sampling and character evolution

When comparing the topologies of the complete and the 50% pruned datasets (Fig. 3.3), I obtained an $E = 0.1677$. As explained before, trees that are identical have $E = 0$, whereas completely different topologies have an $E = 1$. The topology inferred from the dataset in which only two species (*Ikeakogi tayrona* and *Chimerella*

mariaelenae) were excluded is almost identical to the complete tree, the only difference being the position of *Vitreorana eurygnatha*, which is clustered as sister to the *V. antisthenesi + V. castroviejoi* clade.

The distribution of characters in centrolenid frogs is summarized in Appendix 3.4. The reconstruction of character evolution on the three topologies is presented in Table 3.1 and in Figures 3.4 and 3.5. It should be noted that under any of the phylogenetic frameworks or optimality criteria, the estimated history is complex and always implies multiple origins or losses of the six traits under study. Parsimony inference usually results in multiple solutions for any given reconstruction. ML reconstructions mostly agree with parsimony, but, when using the Asymmetrical model, the total number of inferred gains and/or losses can be higher (Table 3.1). The Asymmetrical model, however, is not a significantly better explanation of the data than the 1-parameter (Mk1) model, in which rates of gains and losses are equal (Table 3.2); the only exception is when comparing the inferred evolution of humeral spines when 50% of the species have been removed (Table 3.2). For most of the cases, except for the inference of humeral spine evolution, the Mk1 model and the Asymmetric model yield congruent reconstructions (Table 3.1).

The hypothesized traits of the most recent common ancestor (MRCA) of centrolenids are shown in Table 3.3. For half of the traits examined, the ancestral reconstructions are constant (i.e., transparent hepatic peritoneum, white pericardium, and partially transparent venter) under all scenarios. Also, most reconstructions indicate that the MRCA of Glassfrogs had an opaque visceral peritoneum and

oviposited on the upper sides of leaves (Table 3.3; Fig. 3.1). The ancestral condition of the humerus (with or without a spine) is ambiguous and varies, depending on the taxon sampling and method of reconstruction (Tables 3.1, 3.3).

Character correlation

I found a significant correlation between the evolution of complete ventral transparency and the presence of two character states, a white gastrointestinal peritoneum and a white hepatic peritoneum (Table 3.4). The only exception to this generality is in the case that ventral transparency is reconstructed as having six independent origins, five of which are associated with the presence of a white gastrointestinal peritoneum ($P = 0.11586$; Table 3.4).

DISCUSSION

Effect of incomplete taxon sampling

How much confidence should we place on the reconstructed ancestral characters? As discussed by Cunningham (1999) and Pagel et al. (2004), there are several potential sources of error. I addressed the effect of incomplete taxon sampling on the topology, and, therefore, on the inference of character evolution. Several studies (Lecointre et al., 1993; Hillis, 1996, 1998; Zwickl and Hillis, 2002; but see Rosenberg and Kumar, 2001) concluded that increased sampling of taxa increases overall phylogenetic accuracy. I find that, when comparing the trees inferred from the original dataset and a pruned dataset (50% of the species removed), all the major

clades are identical and that changes are restricted to areas with low nodal support (Fig. 3.3). Also, given that most of the main clades have a uniform morphology, when reconstructing character evolution on these two trees, the results are highly similar (Tables 3.1, 3.3). Therefore, I conclude that, for this particular dataset (ingroup of ~90 taxa and ~ 4360 bp), the topology and interpretation of character evolution remain fairly stable even when 50% of the species are excluded, suggesting that studies with incomplete taxon sampling can result in a good approximation of real patterns, although they can be less accurate.

When excluding two phylogenetically unstable species (*Ikeakogi tayrona* and *Chimerella mariaelenae*), the resulting topology is almost identical to the complete tree. However, there is variation in the way in which the evolution of humeral spines is reconstructed, especially when using the Asymmetric model (Tables 3.1, 3.3). The placement of *I. tayrona* as sister to all other Glassfrogs means that all the traits present in this species tend to be reconstructed as ancestral. When all the species are included, the Asymmetrical model favors an early evolution of humeral spines and multiple subsequent losses (Table 3.1), but when *I. tayrona* is excluded the same model infers multiple independent gains of humeral spines. The somehow obvious conclusion is that the inclusion/exclusion of early divergent species is likely to have an effect on the interpretation of trait evolution.

Effect of optimality criteria

In most of the cases, ML and parsimony recover similar histories of character evolution (Tables 3.1, 3.3). Given that parsimony methods reconstruct the ancestral

character states to minimize the number of characters required to explain the observed pattern in contemporary species, the estimated total number of gains + losses is sometimes higher in ML reconstructions (Table 3.1). However, when changes between character states are rare, the ML reconstructions are the same as parsimony estimates (Table 3.1), as observed by Schluter et al. (1997).

When comparing reconstructions assuming a single rate for both gains and losses (Mk1 model) and different rates of evolution (Asymmetric model), it is apparent that the two models reconstruct the same pattern of character evolution only when the estimated rates of gains and losses of the Asymmetric model are comparable to the single rate of the Mk1 model (Table 3.2). The mentioned concordance is associated to traits that have few changes in the tree.

In this study, I found two examples (evolution of humeral spines and gastrointestinal peritoneum) in which the rates of change estimated by the Asymmetric model were considerably different than the Mk1 rate, producing contradictory scenarios of trait evolution (Tables 3.1, 3.2). The Mk1 model estimates multiple origins of humeral spines and one loss, whereas the Asymmetric model favors 1 gain and multiple losses (Table 3.1); the latter pattern emerges because the estimated rate of losses is about 10 times greater than the rate of gains. In the second trait, the Asymmetric model estimates many more gains of a white gastrointestinal peritoneum than the Mk1 model as a result of higher rate of gains than losses (Table 3.2). When choosing between contradictory alternatives, it is important to consider if the use of a two-parameter model is justified. As mentioned by Pagel (1999), if a two-

parameter model is not significantly better than a one-parameter model, then the latter model should be preferred. In this case, when all species are included, there are no significant differences between the models (Table 3.2). However, in these cases when contradictory reconstructions are evident, the conclusions on trait evolution should be taken with caution.

The ancestral Glassfrog

Given the phylogenetic framework and methods of character reconstruction, the hypothetical common ancestor of Glassfrogs oviposited on the upper sides of leaves and had a partially transparent venter, a white pericardium, a transparent hepatic peritoneum, and an opaque visceral peritoneum (Table 3.3). The reconstruction of humeral spines is ambiguous and varies depending on the optimality criteria and taxon sampling. The ancestral reconstruction is stable under most of the scenarios presented in Table 3.1, indicating that a variation in taxon sampling is not likely to change the interpretation of the results, unless early divergent species remain unsampled. It is important to emphsize that if *Ikeakogi tayrona* is removed from the analysis, the ancestral Glassfrog is reconstructed unambiguously as lacking humeral spines under parsimony and ML optimization criteria. Then, in the hypothetical case that *I. tayrona* was found to be a close relative of species with humeral spines (*Centrolene* and *Espadarana*), the history of the trait would be unequivocal.

Character evolution and correlation in Glassfrogs

The following discussion is based mainly on the results from the complete dataset, which, at the moment, represents the best estimate of phylogeny. The evolution of humeral spines has been complex (Table 3.1), and partially explains the morphological diversity found in the group (Fig. 3.4). Although there is uncertainty as to whether the evolution of humeral spines has been dominated by multiple origins (parsimony and Mk1 reconstructions) or multiple losses (Asymmetric reconstruction), at this point I prefer the former hypothesis because, as discussed above, a simpler model should be preferred when there is no significant support for the two-rates model. Then, given the assumption that humeral spines originated multiple times (6–8 times; Table 3.1), sexual selection theory provides an explanation for its recurrent evolution. Humeral spines occur only in adult male Glassfrogs, which are territorial and known to display complex fighting behavior (Duellman and Savitzky, 1976; Bolivar et al., 1999; Guayasamin and Barrio-Amorós, 2005). It has been suggested that males use their humeral spines during fights (Bolívar et al., 1999). Given that some species (e.g., *Nymphargus griffithsi, Cochranella balionota*) show intraspecific variation in the shape of the *crista ventralis* (Fig. 3.2), and that having spines may provide an advantage during male-to-male territorial fights, the parallel evolution of armaments (humeral spines) through the process of intrasexual competition is likely.

Other characters that have evolved multiple times in centrolenid frogs are white liver, white digestive tract, and complete ventral transparency (Table 3.1). The results from the Concentrated Changes Test (Table 3.4) reject, for most cases, the null

hypothesis that complete transparency has evolved independently from the presence of white iridophores on the liver and digestive tract. The correlation of these traits has biological meaning because internal organs require protection from potentially harmful UV-radiation. A plausible explanation is that the presence of white lining on the visceral and hepatic peritonea allowed the evolution of complete ventral transparency. This scenario presents a mechanism that explains how completely transparent venters evolved; however, as mentioned above, the adaptive significance of partial or complete ventral transparency in Glassfrogs remains unknown.

With regard to oviposition site, the derived state, oviposition on undersides of leaves, has evolved at least four times, but at considerably different evolutionary scales, a relatively ancient origin of the ancestor of *Hyalinobatrachium,* and recent origins in *Teratohyla spinosa, Centrolene notostictum,* and *C. peristictum*. However, the reconstruction of this behavior is restricted to number of available observations, and we still know little about the number of species that are polymorphic for this character (e.g., *Ikeakogi tayrona, Celsiella revocata, Sachatamia albomaculata, Vitreorana eurygnatha;* Señaris and Ayarzagüena, 2005, Lutz, 1947, McCranie and Wilson, 2002, M. Rada pers. comm.). I hypothesize that the location of the egg clutches on the undersides of leaves is adaptively advantageous to the alternate behavioral state (eggs on the upper sides of leaves), because the location might reduce predation and desiccation. Also, given that the derived state is present in early divergent clades (i.e., all species in *Hyalinobatrachium, Celsiella revocata,* and *Ikeakogi tayrona;* Ruiz-Carranza and Lynch, 1991a; Señaris and Ayarzagüena, 2005;

M. Rada pers. comm.), it is possible that the ancestral species that originated the centrolenid clade were already variable in the ovoposition site.

Another behavior that deserves attention is the evolution of parental care. In species in which the eggs are on the underside of leaves, males usually are found on the same leaf (McDiarmid, 1978; Savage, 2002; Señaris and Ayarzagüena, 2005), a behavior not commonly observed in species that deposit their eggs on the upper sides of leaves. The proximity of males and eggs may be indicative of parental care; however, there are few observations to corroborate this assumption. In the only study involving Glassfrogs (McDiarmid, 1978), increased parental care increased survival of egg clutches. Presently, there are too few studies to try to assess a correlation between parental care and oviposition site.

The evolution of a visible red heart (i.e., transparent pericardium; e.g., *H. aureoguttatum;* Fig. 3.1A) in species of the genus *Hyalinobatrachium* is also more complex that originally thought (Ruiz-Carranza and Lynch, 1998). Although the SH test rejects the monophyly of a clade consisting only of species with the derived condition (transparent pericardium), the estimation of the ancestral state in *Hyalinobatrachium* is ambiguous. There are two equally parsimonious interpretations, a single origin of pericardial transparency in the ancestral species that gave rise to the genus *Hyalinobatrachium*, followed by at least three reversals to the primitive character state (white pericardium), or three independent origins of transparency and one reversal. Additionally, several species present both the ancestral and the derived conditions (Señaris and Ayarzaguena, 2005; Guayasamin et

al., 2006; Appendix 3.4), and it is possible that polymorphism is more widespread than currently reported.

CONCLUSIONS

Haldane (1927) once remarked: "My own suspicion is that the universe is not only queerer than we suppose, but queerer than we can suppose." Concordantly, all traits previously hypothesized as having a unique origin in Glassfrogs have evolved and/or been lost multiple times (Table 3.1). Thus, previous taxonomic treatments (Ruiz-Carranza and Lynch, 1991a; Savage, 2002; Cisneros-Heredia and McDiarmid, 2007) that assumed single origins of these complex characters failed to recognize monophyletic taxa. This study reveals not only the intricate history of these traits, but also some of the processes underlying their evolution. I have shown that correlated evolution plays an important role in the origin of complete ventral transparency, and that male-to-male competition may explain the parallel evolution of humeral spines. Although it seems counterintuitive that traits such as humeral spines or complete ventral transparency could originate multiple times, this re-evolution does not imply that the genes and developmental pathways originated the novo. Instead, given that closely relate taxa share most of their genes, it seems more likely that reactivation of silenced genes already present in the group can account for the repeated presence of certain traits over evolutionary time scale (Marshall et al., 1994). Finally, further experimental studies are needed to measure the impact of the behavioral novelty of depositing eggs on the undersides of leaves in the short term survival of populations, and the extend of parental care in Glassfrogs.

CHAPTER 4

ORIGIN AND SPECIATION OF GLASSFROGS

Species diversify by means of the following mechanisms: vicariance, ecological differentiation, or sexual selection. In the tropics, most of the major speciation hypotheses are based on vicariance. For example, in the Refugia Model, continuous habitat becomes fragmented owing to climatic change (Haffer, 1969, 1974; Vanzolini, 1970; Vanzolini and Williams, 1981; Bush, 1994). In the Riverine Model, major rivers isolate populations (Wallace, 1852; Ayres and Clutton-Brock, 1992). In the Vanishing Refugia Model, species are subdivided into a series of refuges and, as refuges decrease in size because of environmental forcing, some populations adapt to and persist within the novel habitat (Vanzolini and Williams, 1981). In the Disturbance Model, allopatric speciation is produced and maintained through intermediate disturbance and habitat heterogeneity (Connell, 1978; Hubbell, 1979; Gentry, 1989; Colinvaux, 1993; Bush, 1994). Vicariant models for the Andes include factors such as linearity of the montane range that produces elongate geographic ranges and reduces potential contact and gene flow among parapatric forms (Remsen, 1984). Lynch and Duellman (1997) proposed that speciation is the result of adaptation to novel climates (resulting from the uplift of the Andes) coupled with fragmentation of the once contiguous habitat. Most of the models that are based on vicariance predict that sister species have allopatric distributions, although the Vanishing Refugia Model can result in sympatric or parapatric species (Vanzolini and Williams, 1981).

Models based on divergent selection across environmental gradients differ from allopatric models in that complete suppression of gene flow is not a prerequisite for phenotypic divergence and speciation (Edler, 1977; Orr and Smith, 1998; Rice and Hostert, 1993; see Moritz et al., 2000). The gradient (or "divergence with gene flow") model predicts that sister taxa should occupy distinct, but adjacent, habitats (Moritz et al., 2000), a prediction shared with the Vanishing Refugia Model, but the latter also requires severe population bottlenecks and range expansion, whereas the gradient model does not (Moritz et al., 2000).

Sympatric speciation may occur through processes such as genome duplication (Evans et al., 2004) and sexual selection (Reynolds and Fitzpatrick, 2007). Under a model of sympatric speciation, the expectation is that sister species are primarily syntopic (Lynch, 1989).

Although allopatry is widely accepted as the most important cause of speciation (Mayr, 1940, 1942; Lynch, 1989), the number of studies that corroborate or reject this null hypothesis is limited (but see Schneider et al., 1999; Ogden and Thorpe, 2002; Graham et al., 2004; Kozak and Wiens, 2006). In this study, questions related to the origin, distribution, and geography of speciation are addressed using Glassfrogs (Centrolenidae) as a model system. This group is appropriate because it is relatively species rich (147 species; AmphibiaWeb, 2006), widely distributed (Neotropics), and a has a relatively well-resolved phylogenetic framework (Fig. 4.1).

The following questions are addressed. (1) What is the geographic origin of the group—Central or South America? (2) How have Glassfrogs obtained their

current distribution? (3) Which speciation model explains the observed distribution patterns of Glassfrog species?

METHODS

Ancestral area reconstruction

To infer the historical biogeography of the Glassfrogs, I applied parsimony reconstructions as implemented in Mesquite 1.05 (squared parsimony option; Maddison and Maddison, 2004) by optimizing area-characters onto the molecular tree. Maximum likelihood reconstruction was not performed because it cannot accommodate polymorphisms. Also, I used the program Dispersal-Vicariance analysis (DIVA; Ronquist, 1996, 1997). However, results in DIVA are not discussed because area optimizations near the root were too ambiguous to be informative. The reconstructions obtained using Mesquite were similar to those from DIVA at the tips of the tree.

Biogeographic areas are modified from those presented by Duellman (1999). Herein, I use the following areas:

Central America.—Although Central America is topographically complex, herein the area is coded as a single biogeographic region for simplicity. The Isthmus of Panama is defined as the limit between Central and South American.

Chocó.—Tropical rainforest (< 800 m) limited by the Pacific Ocean on the west, the

Andes on the east, the Isthmus of Panama on the north, and reaching to about 1˚40' S in Ecuador.

Cordillera de la Costa (Venezuela).—Mountain range parallel to the Caribbean coast of Venezuela, separated from the Cordillera de Mérida by the Turbio-Yaracuy Depression.

Amazonia.—Tropical rainforest (< 800 m) east of the Andes, south of the Amazon River.

Guianan Shield.—Lowlands and highlands corresponding to the Guianan Shield in northeast South America (Gibbs and Barron, 1993). Broadly, this area includes the rainforest north of the Amazon River and east of the Andes.

Northern Andes.—From the Cordillera de Mérida in Venezuela south to the Huancabamba Depression in Peru at elevations greater than 800 m.

Central and Southern Andes.—From the Huancabamba Depression in Peru south the Andes of Bolivia at elevations greater than 800 m.

Atlantic Forest.—Tropical and subtropical moist forest along the Atlantic coast of Brazil from Rio Grande do Norte state in the north to Rio Grande do Sul state in the south, and inland as far as Paraguay and the Misiones Province of Argentina.

Sierra Nevada de Santa Marta.—Restricted to the Sierra Nevada de Santa Marta in

Colombia.

Speciation patterns

To establish patterns of speciation, I coupled the phylogenetic framework shown in Figure 4.1 and the distributions of species (obtained from IUCN et al. 2006; Table 4.1). In general, if the ranges of sister taxa are non-overlapping, the mode of speciation is hypothesized to be allopatric, whereas if sister species are primarily syntopic, sympatric speciation is inferred to be prevalent (Lynch, 1989); parapatric distributions are considered to be the product of speciation via ecological differentiation. Interpretation of speciation patterns assumes that currently recognized species are valid and that taxon sampling is adequate to make general inferences on the mechanisms of cladogenesis. Although this study has incomplete taxon sampling, I assume that the phylogenetic and geographic signals are sufficiently strong to permit a general discussion of the geography of speciation. An additional limitation is that the distribution of Glassfrogs is not well known; thus, in most of the cases, the known ranges probably under represent the real geographic distribution of species. Also, the use of current distributions to infer speciation assumes that range shifts have not randomized the relationship between cladogenetic history and geographic overlap of sister taxa (Barraclough and Vogler, 2000; Losos and Glor, 2003)

RESULTS

Ancestral area reconstruction and speciation patterns

The most parsimonious area reconstruction requires 25 steps and is shown in Figure 4.1. The present distribution of centrolenid frogs is explained in Figure 4.2 and represents a scenario in which Glassfrogs originated in Guianan and dispersed to other regions, including Central America. Table 4.2 summarizes mechanisms of speciation that are concordant with the phylogeny and distribution of Glassfrogs.

Pattern of diversity

The diversity of Glassfrogs reaches its maximum in cloud forests of the northern Andes and decreases as latitude increases (Fig. 4.3). Centrolenid frogs have not been able to colonize several South American ecoregions, including the Andes of Argentina and Chile, Cerrado-Caating-Chaco, Pampean-Monte, Austral Temperate Forest, Patagonia, and Llanos. (For definitions, see Duellman 1999.)

Diversity seems to be associated with topographic complexity. In Colombia, where the Andes are divided into three mountain ranges, the species richness of centrolenid frogs reaches its peak (Fig. 4.3). Although, the number of species per country and per biogeographic area is likely to change, the general pattern of diversity is clear. The Northern Andes is the most diverse region with 77 species, followed by the Central and Southern Andes (17 species), Amazonia (16 species), Guianan (15 species), Chocó (14 species), Central America (14 species), Cordillera de la Costa (8 species), Atlantic forest (3 species), and Sierra Nevada de Santa Marta (1 species).

DISCUSSION

Historical biogeography of Glassfrogs

The first of the questions posed in this study is unambiguously resolved by ancestral area reconstruction. Glassfrogs originated in South America and dispersed at least four different times into Central America (Figs. 4.1, 4.2). Because anurans usually have low dispersal abilities and are not tolerant to salt water (Duellman and Trueb, 1994), the most likely scenario is that Central America was colonized by Glassfrogs once the closure of the Panama Gap was completed (ca. 3 Mya; Coates and Obando, 1996).

An ecological observation is congruent with the South American origin of Glassfrogs. Given that the Darién lowlands separate South and Central America, it is reasonable to hypothesize that only lineages adapted to lowlands would be able to disperse into Central America. The observed pattern is congruent with this hypothesis; only South American clades that inhabit the lowlands of the Amazon Basin and/or the Chocó (*Cochranella, Teratohyla, Sachatamia, Hyalinobatrachium;* Table 4.3) are also found in Central America. In contrast, South America clades restricted to the mountains of the Andes (*Centrolene, Nymphargus, Rulyrana*) and/or Guianan highlands are not found in Central America, which lacks species adapted exclusively to high elevations. Additionally, Central American species are deeply nested in the phylogeny (Fig. 4.1), as expected in a scenario of South American origin.

How have Glassfrogs obtained their current distribution? Although there is not a definitive answer to this question, the distribution pattern is congruent with the scenario summarized in Figures 4.1 and 4.2, and described below. In South America, Guianan is inferred as the ancestral area of Glassfrogs. I caution that this result is influenced by the distribution of the sister species of Centrolenidae (i.e., *Allophryne ruthveni*). When *Allophryne* is excluded from the analysis, the ancestral area of Glassfrogs is ambiguous (Guiana, Sierra Nevada de Santa Marta). Similarly, the inference of the Sierra Nevada as an ancestral area is based solely on the phylogenetic position of *Ikakogi tayrona,* the only Glassfrog that inhabits this ecoregion. Assuming that the topology is correct, the reconstruction suggests that dispersal to Sierra Nevada occurred early in the history of centrolenid frogs. From a geological perspective, the Sierra Nevada is part of the Maracaibo Subplate Realm, which is characterized as the most nothwestern portion of the Guianan Shield (Cediel et al. 2003). Therefore, an ancient dispersal from the Guianan Shield to the Sierra Nevada is plausible.

The proximity of the Cordillera de la Costa made possible dispersal events from the adjacent Guianan Shield, as exemplified by the distribution of *Celsiella, Hyalinobatrachium,* and *Vitreorana*. The presence of a Glassfrogs in the Atlantic Forest is explained by the connection between the Guianan and Brazilian Shields; the most obvious vicariant event separating species from the two areas is the Amazon River, which established a connection to the Atlantic during the Late Miocene (11–5 Ma; Hoorn et al., 1995).

Subsequent dispersal events allowed invasions of Amazonia, Chocó, and Central America, as observed in *Teratohyla* and *Hyalinobatrachium*. Then, the uplift of the Eastern Andean Cordillera (12.9–3.6 Ma; Hoorn et al., 1995; Hooghiemstra et al., 2006) and the Mérida Andes (28.4–23.0 Ma; Kohn et al., 1984) would have made colonization of the Andes from the Cordillera de la Costa a likely event. The mentioned dispersal route is consistent with the geology of the region, being the Cordillera de la Costa older than the Northern Andes (Steyermak, 1979), and is exemplified by the relationships among *H. pallidum, H. ibama, H. duranti, H.* sp., *H. fragile, H. orocostale,* and *H. orientale* where Andean species (*pallidum, ibama, duranti*) are nested within species from the Cordillera de la Costa (*H.* sp., *H. fragile, H. orocostale,* and *H. orientale*).

Another factor that might have facilitated dispersion from the Guianan to adjacent lowlands and then to Northern Andes (which has been colonized at least four independent times; Fig. 4.1) is the lowering of temperatures during glacial periods. Hooghiemstra (1984) suggested that for much of the last northern hemispheric ice age, Andean taxa occupied ranges about 800 m down slope of their present distribution.

Centrolene and *Nymphargus* are endemic to the Andes. These two genera have species from the Northern and Central Andes. The ancestral area reconstruction favors an origin in the north a subsequent dispersal to the south. Interestingly, the genus *Nymphargus* is absent from the Eastern Cordillera, suggesting that it originated in the Central Andes and has been unable to disperse to the Eastern Cordillera. These

Andean clades, although speciose, have not given rise to species restricted to adjacent lowlands (e.g., Amazonia, Chocó, Central America), thereby providing an example of niche conservatism at deep nodes. Also, *Centrolene* and *Nymphargus* are not present in the Cordillera de la Costa, implying that, for these clades, the Turbio-Yaracuy Depression is an important biogeographic barrier.

Hyalinobatrachinae originated in Guiana. From there, the inferred reconstruction suggests dispersals to Northern Andes, Amazonia, Chocó, Central America, and two independent dispersals to Cordillera de la Costa. There are two species currently found on the Amazonian slopes of the Andes (*H. bergeri, H. pellucidum*) that are nested in a clade of otherwise Chocóan and Central American species, suggesting another vicariant event produced by the closure of the Chocóan-Amazonian connection.

In Clade C, ancestral area reconstruction has a similar pattern as that observed in Hyalinobatrachinae, including dispersal to Amazonia, Chocó, and Central America (*Teratohyla, Cochranella, Sachatamia;* Fig. 4.1). Also, this Clade is present in the Andes.

Finally, given the incomplete sampling, other scenarios are possible. For example, an origin from Central Andes should not be discarded. Also, I would like to emphasize that biogeographic scenarios are prone to change when early divergent species are included. This effect is already illustrated in the present study, in which including species such as *Allophryne ruthveni* (sister species of Centrolenidae) and the *Ikakogi tayrona* heavily affect the ancestral area reconstruction (see above). Given

that about 45% of Glassfrogs species are not incorporated in this analysis, this biogeographic interpretation should be considered to be preliminary.

Speciation patterns in Glassfrogs

Are the observed patterns of speciation better explained by vicariant or ecological models of speciation? Comparison between phylogeny and species distribution strongly supports vicariance as the main mechanism producing speciation (Table 4.2) as hypothesized by Lynch (1989). There are only few cases in which sympatric or selection models are necessary to explain the observed distribution of sister species (Table 4.2). These examples, however, should be studied in depth given that current sympatric and parapatric distributions can be the result of range expansions that have occurred after an allopatric speciation event.

Then, in contrast to recent studies that conclude that differential selection played an important role in species differentiation (Schneider et al., 1999; Ogden and Thorpe, 2002; Graham et al., 2004; Hall, 2005), I find that vicariance, possibly linked to niche conservatism, has been the main factor in centrolenid speciation. Some of the major geographic changes that seem to have influenced the distribution of clades in Centrolenidae include the uplift of the Eastern Andean Cordillera, an event that isolated Amazonian from Chocóan species (Hoorn et al., 1995). The Eastern Cordillera formed a continuous range between 12.9–11.8 Ma (Hoorn et al., 1995); however, probably it only became a significant barrier to lowland species during the early Pliocene (5.3–3.6 Ma; Hooghiemstra et al., 2006). Its uplift also produced a

shift in the direction of the Amazon River toward the Atlantic (Hoorn et al., 1995). Once the Andes were colonized by Glassfrogs, river valleys and depressions would be the most common cause of speciation (Table 4.2). Also, it is likely that species were further isolated during climate change in the Quaternary (Hooghiemstra et al., 2006).

Finally, given that different studies have reached a variety of conclusions concerning speciation in the Neotropics, it is reasonable to conclude that dissimilar mechanisms are important in the cladogenesis of distinctive groups; this variation is likely to be associated with the dispersal ability, reproductive mode, and niche breath of organisms. Future studies should test if allopatric sister species in Centrolenidae occupy similar ecological conditions; a result showing overlapping niches would favor the hypothesis that niche conservatism promotes geographic isolation and speciation (Kozak and Wiens, 2006).

CONCLUSIONS

The present study provides the first comprehensive molecular phylogeny of the family Centrolenidae. The inferred topology is highly incongruent with previous hypotheses of centrolenid relationships based on morphological characters.

I formalize the evolutionary proximity of Centrolenidae and Allophynidae with the name Centrolenia. This arrangement maintains the name and species content of the two families included in Centrolenia, avoiding nomenclatural instability.

Based on the molecular topology, a phylogenetic taxonomy of Glassfrogs is proposed. Within Centrolenidae, I recognize two subfamilies and a total of 12 genera, seven of which are new. The methodology used to define the mentioned taxa has the ultimate goal to provide long-term name stability in the family.

The study reveals an intricate history of morphological and behavioral traits, as well as some of the processes underlying their evolution. Each of the traits that has been postulated to trace relationships unambiguously in this group has turned out to have had a complex evolutionary history with multiple origins and/or losses. I have shown that correlated evolution plays an important role in the origin of complete ventral transparency, and that male-to-male competition may explain the parallel evolution of humeral spines. Also, I suggest that complete taxon sampling may not be critical for an accurate reconstruction of character evolution, as long as there is adequate representation of the morphological/behavioral diversity of all major clades.

The biogeographic analysis indicates that Glassfrogs originated in South America and dispersed at least four different times into Central America. Ancestral

area reconstruction indicates that, specifically, centrolenids originated in Guianan and dispersed to adjacent ecoregions. Finally, comparison between phylogeny and species distribution strongly supports vicariance as the main mechanism producing speciation in Glassfrogs.

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TABLES

TABLE 1.1. Genes and primers used in this study. The arrow indicates primers located in the forward (→) or in the reverse (←) strand.

Primer	Sequence (5´ → 3´)	Source
Mitochondrial 12S		
MVZ 59	ATAGCACTGAAAAYGCTDAGATG →	Graybeal (1997)
tRNAphe	ATAGCACTGAAAAYGCTDAGATG →	Goebel et al. (1999)
t-Phe-frog	ATAGCRCTGAARAYGCTRAGATG →	Wiens et al. (2005); modified "MVZ 59" from Graybeal (1997)
t-Val-frog	TGTAAGCGARAGGCTTTKGTTAAGCT ←	Wiens et al. (2005)
tRNAval	GGTGTAAGCGAGAGGCTT ←	Goebel et al. (1999)
MVZ 50	TCTCGGTGTAAGCGAGAAACTT ←	Graybeal (1997)
Mitochondrial 16S		
16SC	GTRGGCCTAAAAGCAGCCAC →	Darst and Cannatella (2004)
16Sbr-H	CCGGTCTGAACTCAGATCACGT ←	Palumbi et al. (1991)
Mitochondrial ND1		
16S-frog	TTACCCTRGGGATAACAGCGCAA →	Wiens et al. (2005)
TMet-frog	TTGGGGTATGGGCCCAAAAGCT ←	Wiens et al. (2005)
Nuclear *c-myc exon 2*		
cmyc1U	GAGGACATCTGGAARAARTT →	Crawford (2003)
cmyc-ex2 R	TCATTCAATGGGTAAGGGAAGACC ←	Wiens et al. (2005)
Nuclear POMC		
POMC-1	GAATGTATYAAAGMMTGCAAGATGGWCCT →	Wiens et al. (2005)
POMC-2	TAYTGRCCCTTYTTGTGGGCRTT ←	Wiens et al. (2005)
Nuclear RAG1		
R1-GFF	GAGAAGTCTACAAAAAVGGCAAAG →	Faivovich et al. (2005)
R1-GFR	GAAGCGCCTGAACAGTTTATTAC ←	Faivovich et al. (2005)

TABLE 1.2. Thermocycling conditions used to amplify mitochondrial and nuclear genes using the polymerase chain reaction (PCR). Protocols were developed by J. W. Fetzner in J. J. Wiens lab (see Wiens et al., 2005).

Gene	Protocol
12S, 16S	1 cycle: 2 min 94°C, 30 s 42°C, 1 min 72°C
	9 cycles: 30 s 94°C, 30 s 42°C, 1 min 72°C
	30 cycles: 30 s 94°C, 30 s 50°C, 1 min 72°C;
	1 cycle: 5 min 72°C
ND1	1 cycle: 2 min 94°C, 30 s 50°C, 1 min 72°C
	10 cycles: 30 s 94°C, 30 s 50°C, 1 min 72°C
	29 cycles: 30 s 94°C, 30 s 58°C, 1 min 72°C;
	1 cycle: 5 min 72°C
c-myc exon 2	1 cycle: 2 min 96°C
	45 cycles: 20 s 96°C, 45 s 54°C, 90 s 72°C
	1 cycle: 7 min 72°C
POMC, RAG1	1 cycle: 2 min 96°C
	45 cycles: 20 s 95°C, 25 s 52°C, 2 min 72°C
	1 cycle: 7 min 72°C

TABLE 1.3. Estimated parameters for Bayesian analyses as calculated by ModelTest 3.7 (Posada and Crandall, 1998). AIC = Akaike information criterion; I = Proportion of invariable sites; Γ = Gamma distributed rate variation among sites.

Gen	Best-fit Model	AIC score	-ln likelihood	I	Γ
12S	GTR + I + G	44091.0	22035.5	0.2793	0.5343
16S	GTR + I + G	32128.3	16054.1	0.3245	0.5572
ND1, 1st position	GTR + I + G	15685.5	7832.7	0.4010	0.6263
ND1, 2nd position	TVM + I + G	4715.1	2348.6	0.5129	0.4237
ND1, 3th position	GTR + G	51013.4	25497.7	0	1.2935
RAG1, 1st position	TIM + G	1485.0	785.5	0	0.2780
RAG1, 2nd position	K81uf + I	1165.6	575.8	0.7578	Equal
RAG1, 3th position	SYM + G	5191.1	2589.6	0	2.3261
c-myc exon 2, 1st position	TVM + G	1772.4	878.2	0	0.2680
c-myc exon 2, 2nd position	TVMef + G	1454.2	722.1	0	0.3437
c-myc exon 2, 3th position	TVM + G	5865.7	2924.9	0	0.8099
POMC, 1st position	K81uf + I + G	3007.8	1496.9	0.3195	0.5752
POMC, 2nd position	HKY + I + G	2733.2	1360.6	0.3261	0.8427
POMC, 3th position	TVM + G	9151.1	4567.5	0	1.5530

TABLE 1.4. Proportion of Parsimony informative (PI) and Invariable characters.

Gen	Alignment positions	No. of PI	Proportion of PI	No. of Invariable sites	Proportion of invariable sites
12S	974	472	0.485	366	0.376
16S	895	376	0.420	384	0.429
ND1	973	533	0.548	370	0.380
c-myc exon 2	430	141	0.328	141	0.481
RAG1	456	152	0.333	152	0.533
POMC	634	251	0.396	251	0.481

TABLE 3.1. Character evolution (gains and losses) in Glassfrogs. Maximum Likelihood (ML) reconstruction was conducted using the Mk1 model (1 parameter) and the Asymmetrical model (2 parameters). Likelihood reconstruction of some traits was not possible because of polymorphisms or missing data on character state.

	All species			Excluding *Centrolene tayrona* and *C. mariaelenae*			Randomly excluding 50% of the species		
	Parsimony	ML (Mk1)	ML (Asymm.)	Parsimony	ML (Mk1)	ML (Asymm.)	Parsimony	ML (Mk1)	ML (Asymm.)
White gastrointestinal peritoneum	4 gains, 5 losses	4 gains, 5 losses; 6 gains, 3 losses	11 gains	4 gains, 5 losses; 6 gains, 3 losses	6 gains, 3 losses	10 gains	4 gains, 3 losses; 5 gains, 2 losses; 7 gains	7 gains, 1 lose	8 gains
White hepatic peritoneum	4 gains, 1 lose	4 gains, 1 lose	4 gains, 1 lose	3 gains, 1 lose	3 gains, 1 lose	3 gains, 1 lose	2 gains, 3 losses; 3 gains, 2 losses; 4 gains, 1 lose	4 gains, 1 lose	4 gains, 1 lose
Transparent pericardium	1 gain, 5 losses; 3 gains, 3 losses; 6 gains	?	?	1 gain, 5 losses; 3 gains, 3 losses; 6 gains	?	?	2 gains, 3 losses 4 gains, 1 lose	?	?
Complete ventral transparency	4 gains, 2 losses; 5 gains, 1 lose; 6 gains	6 gains	6 gains	3 gains, 2 losses; 4 gains, 1 lose; 5 gains	5 gains	5 gains	3 gains, 2 losses; 4 gains, 1 lose; 5 gains	5 gains	5 gains
Eggs on underside of leaves	4 gains	?	?	4 gains	?	?	1 gain	?	?
Humeral spines	6 gains, 3 losses; 7 gains, 2 losses; 8 gains, 1 lose	8 gains, 1 lose	1 gain, 13 losses	6 gains, 1 lose	6 gains, 1 lose	6 gains, 1 lose	2 gains, 4 losses; 6 gains	5 gains, 1 lose	1 gain, 8 losses

TABLE 3.2. Estimated likelihood of the one-parameter model (Mk1; rates of gains and losses are equal) and the two-parameter model (Asymmetric; rates of gains and losses are estimated) for traits in Glassfrogs. In all cases, the likelihoods of the Asymmetric model are better than the Mk1 model, but the difference is significant in only one case. Polymorphic characters are not included. Probability (*P*) is under a Chi-square distribution. RG = estimated rate of gain, RL = estimated rate of loss.

Character state	All species			Excluding *Centrolene tayrona* and *C. mariaelenae*			Randomly excluding 50% of the species		
	Mk1 model	Asymmetric model	P	Mk1 model	Asymmetric model	P	Mk1 model	Asymmetric model	P
White gastrointestinal peritoneum	-logL = 33.801 rate = 1.2963	-logL = 32.152 RG = 2.2429 RL = 0.4125	0.0693	-logL = 32.008 rate = 1.289	-logL = 30.506 RG = 2.163 RL = 0.430	0.0831	-logL = 21.029 rate = 1.731	-logL = 19.483 RG = 2.483 RL = 0.540	0.0787
White hepatic peritoneum	-logL = 22.077 rate = 0.6257	-logL = 21.919 RG = 0.742 RL = 0.4637	0.5743	-logL = 19.587 rate = 0.5393	-logL = 19.566 RG = 0.5819 RL = 0.4808	0.839	-logL = 17.820 rate = 1.082	-logL = 17.7056 RG = 1.3145 RL = 0.8525	0.632
Complete ventral transparency	-logL = 22.865 rate = 0.7494	-logL = 22.268 RG = 0.937 RL = 0.3148	0.2747	-logL = 20.497 rate = 0.667	-logL = 20.130 RG = 0.8191 RL = 0.3285	0.3915	-logL = 16.534 rate = 1.026	-logL = 16.088 RG = 1.2482 RL = 0.5072	0.3277
Humeral spines	-logL = 30.377 rate = 1.1755	-logL = 29.322 RG = 0.4626 RL = 4.654	0.1464	-logL = 26.674 rate = 0.964	-logL = 26.377 RG = 0.8983 RL = 1.993	0.441	-logL = 19.647 rate = 1.370	-logL = 17.5260 RG = 0.4802 RL = 4.105	0.0394*

TABLE 3.3. Hypothesized traits in the most recent common ancestor of Glassfrogs. Maximum Likelihood (ML) reconstruction was conducted using the Mk1 model (1 parameter) and the Asymmetrical model (2 parameters); values represent proportional likelihoods and asterisks indicate that the inferred character state is significantly better the alternative character state. Likelihood reconstruction of some traits was not possible because of polymorphisms or missing data on character state.

	All species			Excluding *Centrolene tayrona* and *C. mariaelenae*			Randomly excluding 50% of the species		
	Parsimony	ML (Mk1)	ML (Asymm.)	Parsimony	ML (Mk1)	ML (Asymm.)	Parsimony	ML (Mk1)	ML (Asymm.)
Visceral peritoneum	Opaque	Opaque = 0.858	Opaque = 0.995*	Ambiguous	Opaque = 0.6368	Opaque = 0.9554*	Opaque	Opaque = 0.839	Opaque = 0.9876*
Hepatic peritoneum	Transparent	Transparent = 0.9941*	Transparent = 0.9965*	Transparent	Transparent = 0.9309*	Transparent = 0.944*	Transparent	Transparent = 0.9499*	Transparent = 0.9745*
Pericardium	White	?	?	White	?	?	White	?	?
Ventral parietal peritoneum	Partially transparent	Partially transparent = 0.9934*	Partially transparent = 0.9983*	Partially transparent	Partially transparent = 0.946*	Partially transparent = 0.9743*	Partially transparent	Partially transparent = 0.9816*	Partially transparent 0.9931*
Egg deposition site	On leaves	?	?	On leaves	?	?	Ambiguous	?	?
Humeral spines	Ambiguous	Absent = 0.798	Present = 0.9512*	Absent	Absent = 0.9865*	Absent = 0.9491*	Ambiguous	Absent = 0.7123	Absent = 0.9499*

TABLE 3.4. Character correlation in Glassfrogs. The evolution of ventral transparency is hypothesized to be dependant on the presence of iridophores on the hepatic and digestive peritonea. Three different scenarios of the origin of complete ventral transparency are presented, representing the ambiguity in the number of times that the trait has been gained and/or lost.

	Evolution of complete ventral transparency		
	6 gains	5 gains and 1 lose	4 gains and 2 losses
White hepatic peritoneum	$P = 0.00277$**	$P = 0.005450$**	$P = 0.00825$**
White gastrointestinal peritoneum	$P = 0.11586$	$P = 0.02716$*	$P = 0.003930$*

TABLE 4.1. Distribution of species included in the area reconstruction analysis. See IUCN et al. (2006) for species maps.

Species	Distribution	Elevation
Allophryne ruthveni	Guiana, Amazonia	0–300 m
Celsiella vozmedianoi	Cordillera de La Costa	750–780 m
Celsiella revocata	Cordillera de La Costa	1200–1800 m
Centrolene altitudinale	Andes (North)	1975–2400 m
Centrolene antioquiense	Andes (North)	1850–2450 m
Centrolene bacatum	Andes (North)	1950–2100 m
Centrolene buckleyi MAR 371	Andes (North)	2100–3100 m
Centrolene buckleyi KU 178031	Andes (North)	2100–3100 m
Centrolene daidaleum	Andes (North)	1630–2060 m
Centrolene garciae	Andes (North)	1900–3030 m
Centrolene geckoideum	Andes (North)	1750–2500 m
Centrolene hesperium	Andes (South)	1500–1800 m
Centrolene hybrida	Andes (North)	1410–2020 m
Centrolene notostictum	Andes (North)	1600–2440 m
Centrolene peristictum	Andes (North)	1350–1820 m
Centrolene pipilatum	Andes (North)	1300–1740 m
Chimerella mariaelenae	Andes (North)	1400–1820 m
Cochranella cf. *savagei*	Andes (North)	1400–2410 m
Centrolene venezuelense	Andes (North)	2100–3050 m
Cochranella litoralis	Choco	150–220 m
Cochranella euknemos	Central America, Choco	100–1650 m
Cochranella mache	Choco	500–645 m
Cochranella nola	Amazonia, Andes (South)	500–1750 m
Cochranella cf. *nola* CBG 1096	Amazonia, Andes (South)	500–1750 m
Cochranella granulosa	Central America	30–1500 m
Hyalinobatrachium aureoguttatum	Choco, Andes (North)	45–1570 m
Hyalinobatrachium aff. *bergeri* MTDD 46305	Andes (South)	1770 m

TABLE 4.1. Continued.

Species	Distribution	Elevation
Hyalinobatrachium bergeri	Amazonia, Andes (South)	300–2000 m
MNCN/AND 5547		
Hyalinobatrachium chirripoi	Central America, Choco (< 200 m)	0–700 m
Hyalinobatrachium colymbiphyllum	Central America, Choco, Andes (North)	0–1800 m
Hyalinobatrachium crurifasciatum	Guiana	300–1850 m
Hyalinobatrachium duranti	Andes (North)	1800–2400 m
Hyalinobatrachium eccentricum	Guiana	1750 m
Hyalinobatrachium fleischmanni	Central America, Choco, Andes (North)	0–1680 m
Hyalinobatrachium fragile	Cordillera de La Costa	100–700 m
Hyalinobatrachium aff. *iaspidiense*	Guiana	25–1000 m
Hyalinobatrachium iaspidiense	Guiana	25–1000 m
Hyalinobatrachium ibama	Andes (North)	1600–2050 m
Hyalinobatrachium ignioculus	Guiana	600 m
Hyalinobatrachium ignioculus	Guiana	600 m
Hyalinobatrachium mondolfii	Guiana	0–100 m
Hyalinobatrachium munozorum	Guiana, Andes (North)	< 960 m
QCAZ 31056		
Hyalinobatrachium cf. munozorum	Amazonia, Andes (South)	200–1840 m
CBG 1099, MNCN 43691		
Hyalinobatrachium nouraguensis	Guiana	50–150 m
Hyalinobatrachium orocostale	Cordillera de La Costa	1500 m
Hyalinobatrachium orientale	Cordillera de La Costa	190–1200 m
Hyalinobatrachium aff. *pellucidum*	Andes (North)	1740 m
Hyalinobatrachium pallidum	Andes (North)	1650–1768 m
MHNLS 17238		
Hyalinobatrachium cf. *pallidum*	Andes (North)	1500 m
MHNLS 17881		
Hyalinobatrachium sp MIZA 317	Cordillera de La Costa	1000 m
Hyalinobatrachium tatayoi	Andes (North)	301 m
Hyalinobatrachium valerioi	Central America, Choco	0–400 m
Hyalinobatrachium vireovittatum	Central America	880–1100 m
Ikakogi tayrona	Sierra Nevada de Santa Marta	980–1790 m

TABLE 4.1. Continued.

Species	Distribution	Elevation
Nymphargus bejaranoi	Andes (South)	1600–2400 m
Nymphargus cochranae	Guiana, Andes (North)	300–1600 m
Nymphargus aff. *cochranae*	Guiana, Andes (North)	300–1600 m
Nymphargus grandisonae	Andes (North)	1140–2710 m
Nymphargus garciae	Andes (North)	1900–3030 m
Nymphargus griffithsi	Andes (North)	1780–2650 m
Nymphargus megacheirus	Andes (North)	1300–1750 m
Nymphargus mixomaculatus	Andes (South)	2625–2750 m
Nymphargus pluvialis	Andes (South)	1820–2000 m
Nymphargus aff. *posadae*	Andes (North)	2690 m
Nymphargus posadae	Andes (North)	1100–2800 m
Nymphargus rosada	Andes (North)	1100–2000 m
Nymphargus siren	Andes (North)	1310–1700 m
Nymphargus wileyi	Andes (North)	2100 m
Rulyrana adiazeta	Andes (North)	1130–2060 m
Rulyrana flavopunctata	Andes (North)	300–1000 m
Rulyrana puyoensis	Guiana, Andes (North)	400–1050 m
Rulyrana spiculata	Andes (South)	1000–1700 m
Rulyrana cf. *spiculata*	Andes (South)	1000–1700 m
Rulyrana susatamai	Andes (North)	400–1650 m
Sachatamia albomaculata	Central America, Choco, Andes (North)	20–1500 m
Sachatamia punctulata	Andes (North)	500–930 m
Sachatamia ilex	Central America, Choco, Andes (North)	0–1420 m
Espadarana andina	Andes (North)	840–2500 m
Espadarana callistomma	Choco	77–500 m
Espadarana prosoblepon	Central America, Choco, Andes (North)	0–1500 m
Espadarana sp MHUA 4099	Andes (North)	1730 m
Vitreorana antisthenesi	Cordillera de La Costa	220–1200 m
Vitreorana castroviejoi	Cordillera de La Costa	550–800 m
Vitreorana eurygnatha	Atlantic forest	0–1700 m
Vitreorana gorzulai	Guiana	1000–1900 m
Vitreorana helenae	Guiana	700–1000 m

TABLE 4.1. Continued.

Species	Distribution	Elevation
Vitreorana lema	Guiana	1250 m
Vitreorana oyampiensis	Guiana	90–900 m
Vitreorana papillahallica	Guiana	610 m
Teratohyla cf. amelie	Amazonia, Guiana	600 m
Teratohyla midas	Amazonia, Guiana	190–900 m
Teratohyla pulverata	Central America, Choco (< 300 m)	0–960 m
Teratohyla spinosa	Central America, Choco	0–560 m

TABLE 4.2. Hypothesized models explaining speciation between sister taxa.

Sister taxa	Model	Isolating Barrier
Genus *Centrolene*		
altitudinale/notostictum	Vicariance	Táchira Depression
antioquiense/peristictum	Vicariance	Río Cauca Valley
buckleyi/venezuelense	Vicariance	Táchira Depression
buckleyi-venezuelense/hesperium	Vicariance	Huancabamba Depression
daidaleum/savagei	Vicariance	Río Madgalena Valley
hybrida/pipilatum	Vicariance	Linearity of the Andes
Genus *Cochranella*		
euknemos/mache	Environmental selection	Habitat heterogeneity
Genus Nymphargus		
posadae/bejaranoi	Vicariance	Huancabamba Depression, Linearity of the Andes
Genus *Rulyrana*		
flavopunctata/spiculata	Vicariance	Huancabamba Depression, Linearity of the Andes
Genus *Teratohyla*		
amelie/pulverata	Vicariance	Eastern Cordillera (Andes)
midas/spinosa	Vicariance	Eastern Cordillera (Andes)
Genus *Vitreorana*		
helenae-oyampiensis/eurygnatha	Vicariance	Amazon river
Genus *Celsiella*		
revocata/vozmedianoi	Vicariance	Unare Depression
Genus *Hyalinobatrachium*		
aureoguttatum/valerioi	Sympatric	?
pallidum/ibama	Vicariance	Táchira Depression
sp/duranti-pallidum-ibama	Vicariance	Turbio-Yaracuy Depression
fragile/orocostale	Vicariance	Tuy, Valencia, Caracas Valleys
fragile-orocostale/orientale	Vicariance	Unare Depression

TABLE 4.2. Continued.

Sister taxa	Model	Isolating Barrier
chirripoi/colymbiphyllum	Selection/ Sympatric	Habitat heterogeneity, ?
pellucidum/chirripoi-colymbiphyllum	Vicariance	Eastern Cordillera (Andes)

TABLE 4.3. Species found in the Choco and Central America. *Species that have been recently described (after 1995) and their distributions are poorly known.

Species	Central America	Choco (South America)
Cochranella euknemos	+	+
Cochranella granulosa	+	-
**Cochranella litoralis*	-	+
**Cochranella mache*	-	+
Hyalinobatrachium aureoguttatum	+	+
Hyalinobatrachium chirripoi	+	+
Hyalinobatrachium talamancae	+	-
Hyalinobatrachium valerioi	+	+
Hyalinobatrachium vireovittatum	+	-
Sachatamia albomaculata	+	+
Sachatamia ilex	+	+
Espadarana prosoblepon	+	+
**Espadarana callistommum*	-	+
Teratohyla pulverata	+	+
Teratohyla spinosa	+	+

APPEDIXES

APPENDIX 1.1. Ingroup sampling listing species, voucher numbers, localities, and GenBank accession numbers of the sequences analyzed in this study (GenBank Accession Number to be added upon acceptance of the paper). Sequences that were obtained from Genbank are shown in bold.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S (~949 bp)	16 S (~882 bp)	ND1 (961 bp)	POMC (616 bp)	*cmyc-ex* 2 (~406 bp)	Rag-1 (456 bp)
Centrolene andinum	JMG 366	Venezuela: Estado de Mérida: Quebrada Azul (08°41'13" N, 71°29'55" W).	x	x	x	x	x	x
Centrolene altitudinale	MHNLS 17194	Venezuela: Estado Mérida: Quebrada Albarregas (08°37' N, 71°09' W; 2100 m).	x	x	x	x	x	—
Centrolene altitudinale	MHNLS 17225	Venezuela: Estado Mérida: Quebrada Albarregas (08°37' N, 71°09' W; 2100 m).	x	x	x	—	—	x
Centrolene antioquiense	NRPS 014	Colombia: Departamento Antioquia: Vereda El Roble, bosque de la Forzosa, 2127 m.	x	x	x	x	x	x
Centrolene bacatum	QCAZ 22728	Ecuador: Provincia Napo: Yanayacu Biological Station (00º41' S, 77º53' W; 2100 m).	x	x	x	x	x	x
Centrolene callistommum	QCAZ 28555	Ecuador: Provincia Esmeraldas: San Francisco de Bogotá, 83 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc exon 2*	Rag-1
Centrolene buckleyi	KU 178031	Ecuador: Provincia Imbabura: Near Lago Cuicocha (00º18'09" N, 78°36'67'' W; 3010 m).	x	x	x	x	x	—
Centrolene buckleyi	MAR 371	Colombia: Departamento Cundinamarca: Parque Nacional Chingaza, 3035 m.	x	x	x	x	x	x
Centrolene geckoideum	KU 178015	Ecuador: Provincia Pichincha: 1 km SW San Ignacio, 1920 m.	x	x	x	—	—	x
Centrolene gorzulai	MHNLS 16036	Venezuela: Estado Bolívar: Parque Nacional Canaima, Cuenca alta del río Cucurital, Atapare, (05°42' N, 62°33' W).	x	x	x	x	x	x
Centrolene grandisonae	QCAZ 22310	Ecuador: Provincia Pichincha: Mindo Biology Station, 1600 m.	x	x	x	x	x	x
Centrolene hesperium	MHNSM 25802	Peru: Departamento Cajamarca: Quebrada Chorro Blanco.	x	x	x	—	x	x
Centrolene hybrida	MAR 347	Colombia: Departamento Boyacá: Municipio Garagoa: Reserva Natural El Secreto: Quebrada Las Palmitas, 2000 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc exon* 2	Rag-1
Centrolene ilex	UCR 16861	Costa Rica: Provincia Limón.	x	x	x	x	x	X
Centrolene lema	KU 181128	Venezuela: Estado Bolívar: km 127 on the El Dorado-Santa Elena de Uairén road, 860 m.	x	x	x	x	x	X
Centrolene litorale	QCAZ 27693	Ecuador: Provincia Esmeraldas: Stream near Durango, 220 m.	**x**	x	x	x	x	X
Centrolene papillahallicum	BPN 1193	Guyana: Cuyuni-Mazaru Distrit: Upper Partang River.	x	x	x	x	x	X
Centrolene mariaelenae	QCAZ 31729	Ecuador: Provincia Tungurahua: Río Negro.	x	x	x	x	x	X
Centrolene notostictum	MAR 510	Colombia: Departamento Norte de Santander: Piritama, 1800 m	x	x	x	x	x	X
Centrolene peristictum	QCAZ 22312	Ecuador: Provincia Pichincha: Mindo Biology Station, 1600 m.	x	x	x	x	x	X
Centrolene pipilatum	KU 178154	Ecuador: Provincia Napo: Río Salado, 1 km upstream from Río Coca, 1420 m.	x	x	x	—	—	x
Centrolene prosoblepon	MVZ 149741	Costa Rica: Provincia Puntarenas: Monteverde.	—	—	AY819466	AY819085	AY819170	—

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Centrolene prosoblepon	UCR 17102	Costa Rica: Provincia Cartago: Bajos de Cachí (09˚50'2.4" N; 83˚48'22.32" W; 1010 m).	x	x	—	—	—	—
Centrolene sp	MHUA 4099	Colombia: Departamento Antioquia: Finca El Chaquiral.	—	x	x	x	—	x
Centrolene tayrona	MAR 544	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta.	x	x	x	x	x	x
Centrolene tayrona	MAR 545	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta.	x	x	x	x	x	x
Centrolene tayrona	MAR 546	Colombia: Departamento Magdalena, Sierra Nevada de Santa Marta.	x	x	x	x	x	x
Centrolene venezuelense	MHNLS 16497	Venezuela: Estado Mérida: Cordillera de Mérida.	x	x	x	—	x	x
Centrolene venezuelense	EBRG 5244	Venezuela: Estado Mérida: Páramo de Maraisa, 2450 m.	x	x	x	x	—	x
Cochranella adiazeta	MAR 483	Colombia: Departamento Santander: Municipio Charala: Vereda El Reloj.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Cochranella albomaculata	USNM 534151	Honduras: Departamento Gracias a Dios: Quebrada Machin, 540 m.	x	x	x	x	x	x
Cochranella cf. *amelie*	MHNC 5646 / ADN 20619	Peru: Departamento Cusco: Provincia Ouspicanchis: Stream 10 km from Quincemil towards Puerto Maldonado.	x	x	x	x	x	x
Cochranella castroviejoi	MHNLS 16446	Venezuela: Estado Sucre: Península de Paria, 2.5 km W and 3.2 km N of Macuro.	x	x	x	x	x	x
Cochranella daidalea	MHUA 3271	Colombia: Departamento Cesar: Municipio González: Vereda San Cayetano.	x	x	x	x	x	x
Cochranella euknemos	CH 5109	Panama: Provincia Coclé: Cerro Escaliche, Quebrada Escaliche.	x	x	x	x	—	x
Cochranella flavopunctata	QCAZ 32265	Ecuador: Provincia Morona Santiago: 7.6 W of 9 de Octubre (02°13'30.5" S, 78°17'25.6" W; 1715 m).	x	x	x	x	x	x
Cochranella granulosa	CH 5121	Panama: Provincia Coclé: Quebrada Guabalito, Palmarazo, Parque Nacional Omar Torrijos.	x	x	—	—	—	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Cochranella granulosa	USNM 559082	Honduras: Departamento Gracias a Dios: Rus Rus.	x	—	x	x	x	x
Cochranella helenae	MHNLS 17128	Venezuela: Estado Bolivar: Quebrada de Jaspe.	x	x	x	—	—	x
Cochranella helenae	MHNLS 17139	Venezuela: Estado Bolivar: Salto Karuay (05°41'27" N, 61°51'40" W; 900 m).	x	x	x	x	x	x
Cochranella mache	QCAZ 27747	Ecuador: Provincia Esmeraldas: Río Balthazar.	x	x	x	x	x	x
Cochranella midas	KHJ	Ecuador: Provincia Napo: Jatun Sacha, 450 m.	x	x	x	x	x	x
Cochranella nola	CBG 1094	Bolivia: Departamento Cochabamba: Fatima, 700 m.	x	x	x	x	—	x
Cochranella nola	CBG 814	Bolivia: Departamento La Paz: Boquerón (15°36'63" S, 67°20’60" W; 1000 m)	x	x	x	x	x	x
Cochranella oyampiensis	MB 165	French Guiana: Terrain Comté	—	x	—	—	—	—
Cochranella oyampiensis	MB 292	French Guiana: Cayenne: Aya, Trinité	x		x	x	x	x
Cochranella pluvialis	KU 173224	Peru: Departamento Cusco: Rio Umasbamba, 1820 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Cochranella punctulata	MHUA 4071	Colombia: Departamento Antioquia: Municipio de Maceo: Vereda Las Brisas, Hacienda Santa Bárbara.	x	x	x	x	x	x
Cochranella puyoensis	DFCH-USFQ D285	Ecuador: Provincia Napo: 45 E of Narupa, on the Hollín–Loreto road, 800 m.	x	—	—	—	—	x
Cochranella revocata	MHNLS 17319	Venezuela: Estado Aragua: Colonia Tovar.	x	x	x	x	x	x
Cochranella cf. *spiculata*	CBG 806	Bolivia: Departamento La Paz: Boquerón (15°36'63'' S, 67°20'60'' W; 1000 m).	x	x	x	x	x	x
Cochranella spiculata	MHNSM 24867	Peru: Departamento Junin: Provincia Satipo: Distrito Llaylla: Vista Alegre, 1340 m.	x	x	x	x	x	x
Cochranella spinosa	USNM 538863	Honduras: Departamento Olancho: Quebrada El Guasimo (14°35' N, 85°18' W; 140 m).	x	x	x	x	x	x
Cochranella susatamai	MAR 337	Colombia: Departamento Tolima: Vereda El Tutumo: Quebrada El Coral, 1100 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Cochranella cf. *savagei*	MHUA 4094	Colombia: Departamento Antioquia: Municipio Anorí: Vereda El Retiro.	x	x	x	x	x	x
Cochranella sp	CBG 1096	Bolivia: Departamento Cochabamba: Repechón, 500 m	x	x	x	x	x	x
Hyalinobatrachium aureoguttatum	QCAZ 32105	Ecuador: Provincia Esmeraldas: 2 km E San Francisco.	x	x	x	x	x	x
Hyalinobatrachium antisthenesi	MHNLS 17909	Venezuela: Estado Aragua: Parque Nacional Henri Pittier.	x	x	x	x	x	x
Hyalinobatrachium aff. *bergeri*	MTD 46305	Peru: Departamento Pasco: km 34 on the Oxapampa–Yaupi road.	x	x	x	x	x	x
Hyalinobatrachium bergeri	MNCN/ADN 5547	Peru: Deptartamento Cusco: 6.1 km from Puente Fortaleza towards Quince Mil.	x	x	x	x	x	x
Hyalinobatrachium chirripoi	USNM 538586	Honduras: Departamento Olancho: Quebrada El Guasimo (14°35' N, 85°18' W; 140 m).	x	x	x	x	x	x
Hyalinobatrachium chirripoi	UCR 17424	Costa Rica: Provincia Limón: Aguas Zarcas, Cuenca del Río Banano.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Hyalinobatrachium colymbiphyllum	UCR 17423	Costa Rica: Provincia Puntarenas: Monteverde.	x	x	x	x	x	x
Hyalinobatrachium crurifasciatum	MHNLS 16475	Venezuela: Estado Bolívar: 13 km S Las Claritas.	x	x	x	x	x	x
Hyalinobatrachium duranti	MHNLS 16493	Venezuela: Estado Mérida: La Azulita, 2100 m.	x	x	x	x	x	x
Hyalinobatrachium eccentricum	MHNLS 17335	Venezuela: Estado Bolívar: Top of Auyan-tepui, 1800 m.	x	x	x	—	—	x
Hyalinobatrachium eurygnathum	CFBH 5729	Brazil: Estado Minas Gerais: Itamontes.	AY843595	AY843595	JF 689	—	—	AY844383
Hyalinobatrachium fleischmanni	USNM 559092	Honduras: Departamento Gracias a Dios: Rus Rus.	x	x	x	x	x	x
Hyalinobatrachium fleischmanni	QCAZ 22303	Ecuador: Provincia Esmeraldas: La Tola, 31 m.	x	x	x	x	x	x
Hyalinobatrachium fragile	MHNLS 17161	Venezuela: Estado Cojedes: Road Manrique-La Sierra.	x	x	x	x	x	x
Hyalinobatrachium aff. *iaspidiense*	MB 247	French Guiana: Crique Wapou.	x	x	—	—	x	x
Hyalinobatrachium iaspidiense	MHNLS 17126	Venezuela: Estado Bolivar: Quebrada de Jaspe.	x	x	x	—	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Hyalinobatrachium ibama	MAR 503	Colombia: Departamento de Santander: Municipio Playa de Belén: Qb. Piritama, 1780 m.	x	x	x	x	x	x
Hyalinobatrachium ignioculus	BPN 1315	Guyana: Cuyuni-Mazaru Distrit: Upper Partang River.	x	x	x	x	x	x
Hyalinobatrachium aff. *ignioculus*	SMNS 12251	Guyana: Upper Demerara–Berbice Distrit: Mabura Hill Forest Reserve, Maiko creek.	x	x	x	—	—	x
Hyalinobatrachium mondolfii	MHNLS 17119	Venezuela: Delta Amacuro: Slopes of Serranía de Imatáca, first stream of Caño Acoima, tributary of río Grande.	x	x	x	x	x	x
Hyalinobatrachium aff. *mondolfii*	MB 254	French Guiana: Cayenne: Rivière de Kaw.	—	x	—	—	—	—
Hyalinobatrachium aff. *mondolfii*	MB 260	French Guiana: Crique Gabrielle	x	—	x	x	x	x
Hyalinobatrachium munozorum	QCAZ 31056	Ecuador: Provincia Zamora Chinchipe: Destacamento Militar Shaime, 920 m.	x	x	x	x	—	x
Hyalinobatrachium aff. *munozorum*	CBG 1099	Bolivia: Departamento Cochabamba: Repechón, 500 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Hyalinobatrachium aff. *munozorum*	MNCN 43691	Peru: Departamento Puno: Provincia Sandia: Between St. Rosa y San Juan del Oro.	x	x	x	—	—	x
Hyalinobatrachium nouraguensis	SMNS 12247	Guyana: Upper Demerara–Berbice Distrit: Mabura Hill Forest Reserve, Maiko creek, 60 m.	x	x	x	—	—	x
Hyalinobatrachium orocostale	MHNLS 17247	Venezuela: Estado Guárico: Cerro Platillón, southern slope, Hacienda Picachito, main creek, 1500 m.	x	x	x	x	x	x
Hyalinobatrachium orientale	MHNLS 17878	Venezuela: Estado Sucre: Península de Paria, Cerro Humo (10°41' N, 61°37' W; 850 m).	x	x	x	x	x	x
Hyalinobatrachium cf. *pallidum*	MHNLS 17881	Venezuela: Estado Barinas: San Isidro, 1500 m.	x	x	x	x	x	x
Hyalinobatrachium pallidum	MHNLS 17238	Venezuela: Estado Táchira: Road from Sabana Grande to La Grita, Quebrada Guacharaquita (08°10'02.8" N; 71°58'44.2" W; 1650 m).	x	x	x	—	—	x
Hyalinobatrachium cf. *pellucidum*	QCAZ 29438	Ecuador: Provincia de Morona Santiago: km 6.6 on the Limón–Macas road.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes				Nuclear genes	
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Hyalinobatrachium pulveratum	USNM 538588	Honduras: Departamento Olancho: Matamoros, 150 m.	x	x	x	x	x	x
Hyalinobatrachium sp	MIZA 317	Venezuela: Estado Aragua: Parque Nacional Henri Pittier, Estación Biológica Rancho Grande, 1000 m.	x	x	x	—	x	x
Hyalinobatrachium tatayoi	MHNLS 17174	Venezuela: Estado Zulia: stream near Tokuko.	x	x	x	x	x	x
Hyalinobatrachium taylori	MHNLS 17141	Venezuela: Estado Bolivar: Salto Karuay (05°41'27" N, 61°51'40" W; 900 m).	x	x	x	x	x	x
Hyalinobatrachium valerioi	UCR 17418	Costa Rica: Provincia Puntarenas: Rincón de Osa.	x	x	x	x	x	x
Hyalinobatrachium vireovittatum	CH 5330	Panama: Provincia Coclé: Río Indio.	x	x	x	x	x	x
Nymphargus bejaranoi	CBG 1488	Bolivia: Departamento Cochabamba: Chaquisacha.	x	x	x	x	x	x
Nymphargus cochranae	QCAZ 31113	Ecuador: Provincia Napo: Pacto Sumaco, 1400 m.	x	x	x	x	x	x
Nymphargus aff. cochranae	QCAZ 31340	Ecuador: Provincia Zamora Chinchipe: Estación Cientifica San Francisco (03°58' S, 79°04' W).	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Nymphargus garciae	KU 202796	Ecuador: Provincia Sucumbíos: 18 km E Santa Bárbara, 2550 m.	AY326022	AY326022	—	—	—	—
Nymphargus cf. griffithsi	KU 202801	Ecuador: Provincia Carchi: ~5 km W La Gruel, 2340 m.	AY326025	AY326025	—	—	—	—
Nymphargus griffithsi	QCAZ 31768	Ecuador: Provincia Imbabura: Reserva Biológica Alto Chocó.	x	x	x	x	x	x
Nymphargus megacheira	KU 143272	Ecuador: Provincia Napo: 16.5 km NNE Santa Rosa, 1700 m.	x	x	x	x	x	x
Nymphargus mixomaculata	MTD 45200	Peru: Departamento Huánuco: Provincia Huánuco: Cordillera Carpish, Caserío Carpish de Mayobamba, 2625 m.	—	x	x	x	x	x
Nymphargus aff. *posadae*	AAV 119	Colombia: Departamento Santander: Santuario de Fauna y Flora Guanentá, 2650 m.	x	x	x	x	x	x
Nymphargus posadae	QCAZ 26023	Ecuador: Provincia Napo: Yanayacu Biological Station (00º41' S, 77º53' W; 2100 m).	—	—	—	—	—	x
Nymphargus rosada	MHUA 4308	Colombia: Departamento Antioquia: Municipio Anorí: Finca El Chaquiral, 1732 m.	x	x	x	x	x	x

APPENDIX 1.1. Continued.

Species	Voucher	Locality	Mitochondrial genes			Nuclear genes		
			12 S	16 S	ND1	POMC	*cmyc-ex* 2	Rag-1
Nymphargus siren	KU 179171	Ecuador: Provincia Napo: 3.2 km NNE Oritoyacu (00º27' S, 77º52' W; 1910 m).	x	x	x	x	x	x
Nymphargus vozmedianoi	MHNLS 17877	Venezuela: Estado Sucre: Península de Paria, Cerro Humo (10°42' N, 62°37' W; 800 m).	x	x	x	x	x	x
Nymphargus wileyi	QCAZ 27435	Ecuador: Provincia Napo: Yanayacu Biological Station (00º41' S, 77º53' W; 2100 m).	x	x	x	x	x	x

APPENDIX 1.2. Outgroups included in this study. All sequences were obtained from GenBank, except sequences shown in bold. Family names follow Frost et al. (2006). *See text for suggested changes that apply to marsupial frogs.

Species	Gene Region					
	12S	16S	ND1	Rag1	*cmyc-ex* 2	POMC
Allophrynidae						
Allophryne ruthveni	AY819328	x	AY819458	x	AY819162	AY819077
Amphignathodontidae*						
Flectonotus fitzgeraldi	AY819355	DQ679381	AY819486	DQ679274	AY819189	AY819104
Gastrotheca marsupiata	AY819356	DQ679397	AY819487	DQ679289	AY819190	AY819105
Brachycephalidae						
Oreobates quixensis	—	DQ679380	AY819474	—	AY819178	AY819093
Pristimantis curtipes	AY819343	DQ679379	AY819473	DQ679272	AY819177	AY819092
Bufonidae						
Atelopus peruensis	AY819329	—	AY819459	—	AY819163	AY819078
Dendrophryniscus minutus	AY819332	—	AY819462	DQ503337	AY819166	AY819081

APPENDIX 1.2. Continued.

Species	Gene Region					
	12S	16S	ND1	Rag1	*cmyc-ex* 2	POMC
Calyptocephalellidae						
Caudiverbera caudiverbera	AY819341	DQ872913	AY819471	—	AY819175	AY819090
Ceratophryidae						
Ceratophrys cornuta	AY819342	DQ679376	AY819472	DQ679269	AY819176	AY819091
Lepidobatrachus laevis	AY819345	DQ679377	AY819475	DQ679270	AY819179	AY819094
Telmatobius truebae	AY819348	—	AY819478	DQ679271	AY819182	AY819097
Cryptobatrachidae*						
Stefania evansi	AY819359	DQ679416	AY819490	DQ679307	AY819193	AY819108
Dendrobatidae						
Allobates trilineatus	AY819339	DQ502118	AY819469	DQ503290	AY819173	AY819088
Hyloxalus nexipus	AY819340	AY364553	AY819470	DQ503285	AY819174	AY819089
Hemiphractidae*						
Hemiphractus proboscideus	AY819358	DQ679413	AY819489	DQ679304	AY819192	AY819107

APPENDIX 1.2. Continued.

Species	Gene Region					
	12S	16S	ND1	Rag1	*cmyc-ex* 2	POMC
Hylidae						
Acris crepitans	AY819360	—	AY819491	—	AY819194	AY819109
Agalychnis spurrelli	AY819401	—	AY819532	—	AY819236	AY819151
Anotheca spinosa	AY819361	DQ830813	AY819492	—	AY819195	AY819110
Dendropsophus nanus	AY819373	—	AY819505	AY844437	AY819208	AY819123
Duellmanohyla soralia	AY819362	—	AY819493	AY844378	AY819196	AY819111
Hyla cinerea	AY819366	—	AY819498	AY323766	AY819201	AY819116
Hypsiboas boans	AY819364	—	AY819496	—	AY819199	AY819114
Litoria manya	AY819397	—	AY819529	—	AY819232	AY819147
Phyllomedusa tomopterna	AY819404	—	AY819535	AY844497	AY819239	AY819153
Pseudis paradoxa	AY819353	—	AY819483	AY323773	AY819187	AY819102
Scarthyla goinorum	AY819389	—	AY819521	AY844514	AY819224	AY819139
Scinax crospedospilus	AY819391	—	AY819523	—	AY819226	AY819141
Smilisca fodiens	AY819387	AY843743	AY819519	—	AY819222	AY819137
Sphaenorhynchus lacteus	AY819394	—	AY819526	AY844527	AY819229	AY819144

APPENDIX 1.2. Continued.

Species	Gene Region					
Leiuperidae						
Physalaemus cuvieri	AY819347	AY843729	AY819477	AY844499	AY819181	AY819096
Leptodactylidae						
Leptodactylus didymus	AY819346	—	AY819476	—	AY819180	AY819095
Microhylidae						
Gastrophryne carolinensis	AY819349	—	AY819479	—	AY819183	AY819098
Pipidae						
Xenopus laevis	M27605	NC001573	NC001573	L19324	AY819160	AY819075
Ranidae						
Lithobates catesbeianus	AY819354	—	AY819484	—	AY819188	AY819103
Scaphiopodidae						
Spea bombifrons	AY819327	—	AY819457	—	AY819161	AY819076

APPENDIX 2.1. Specimens examined.

Allophryne: ruthveni, KU 166713, 167756.

Celsiella: revocata, MHNLS 13352, 17319 (topotypes); *vozmedianoi,* MHNLS 13355 (holotype), 16427, 16430, 17877 (topotypes).

Centrolene: altitudinale MHNLS 17194, 17225 (topotypes); *antioquiense* ICN 19649, *bacatum*, KU 202807–12 (paratypes), 170116; *buckleyi*, KU 118006, 148429–30, 155481, 155483, 155485, 164505, 164509–11, 164513, 164515, QCAZ 22388, 26031–32; *fernandoi*, KU 211771–75 (paratypes); *geckoideum*, MNCN 1596 (holotype); KU 164490, 164492, ICN 5598; *gemmatum*, MCZ A-104074, A-104077; *guanacarum*, ICN 11685; *heloderma*, KU 164714–15; *hesperium*, FMNH 232502; *lemniscatus*, KU 217300 (holotype); *lynchi*, KU 164692–99 (paratypes); *paezorum*, ICN 11866; *peristictum*, KU 118051–52; *pipilatum*, KU 143279–82, 143286 (paratypes); *scirtetes*, KU 202720 (holotype); *sanchezi*, ICN 24294; *solitaria*, ICN 24298; *venezuelense* ULABG 2096–9.

"Centrolene": audax, KU 143290, 143292 (paratypes); *ballux*, KU 164725–27; *huilense*, KU 169720–47; *muelleri*, KU 217301 (holotype); *quindianum*, ICN 24920.

Chimerella: mariaelenae, QCAZ 21252, 22363, 31729.

Cochranella: euknemos, KU 77534; *granulosa* SMF 78562, 82896; *litoralis*, ICN 13821, QCAZ 27693; *mache*, QCAZ 22412 (holotype), 31327; *nola* MNCN 42682; *resplendens*, KU 118053 (holotype);

"Cochranella": balionota, KU 164703–11 (paratypes), ICN 13106; *duidaena* MHNLS 12000 (holotype) *megistra,* ICN 17243; *riveroi* MBUCV 6190 (holotype); *xanthocheridia,* ICN 27757.

Espadarana: andina, MHNLS 16485–92 (topotypes); *callistomma,* QCAZ 28555–57 (paratypes), 28803; *prosoblepon,* SMF 3756 (holotype), KU 291165–75, 132462.

Hyalinobatrachium: aureoguttatum, QCAZ 32069–70; *bergeri,* KU 162256; *chirripoi,* KU 36866–70 (paratypes); *colymbiphyllum,* KU 103819; *crurifasciatum* MBUCV 6828 (paratype); *duranti,* MHNLS 16493; *eccentricum* EBRG 3049 (holotype); *fleischmanni,* SMF 3760 (holotype), QCAZ 32107; *fragile,* MHNLS 17161 (topotype); *guairarepanensis* MHNLS 13731(holotype); *iaspidiense,* EBD 28803 (holotype); *ibama,* ICN 50091–92; *ignioculus,* UTA 51658 (paratype), UTACV A51660; *lemur,* KU 211769 (paratype); *mondolfii* MHNLS 12710 (holotype); *munozorum,* KU 155497; *nouraguense,* MNHNP 1999-8604 (holotype)*; orientale,* KU 167371; *orocostale* MHNLS 15108–9 (paratopotypes); *pallidum* MHNLS 17238 (topotype); *pellucidum,* KU 143298 (holotype); *petersi* BM 1902-5-27-24 (holotype); *tatayoi* MHNLS 17174 (holotype); *taylori* BMNH 1939.1.1.65 (holotype); *uranoscopum,* KU 74310–11, 93229–30; *valerioi,* KU 178091.

Ikakogi: tayrona, KU 169750–52, 169754.

Nymphargus: anomalus, KU KU 143299 (holotype); *armatus,* ICN 25000; *bejaranoi,* KU 182370–71 (paratypes); *cariticommatus,* KU 202806 (holotype), 202805 (paratype); *chancas,* KU 211778 (holotype); *cristinae,* ICN 18649; *cochranae,* BM 1912-

11-1-68 (holotype), KU 121033–35, 123218, QCAZ 31113; *grandisonae*, BM 1910-7-11-68 (holotype); KU 164686–690; *griffithsi*, BM 1940-2-20-4 (holotype), 1940-2-20-3 (paratype); KU 142649, 164519–76, 173116, 288992, 188148; *ignotus*, KU 209763–65 (paratypes); *megacheirus*, KU 143246–71 (paratypes); *mixomaculatus*, MHNSM 18653 (holotype); *pluvialis*, KU 173488; *ocellatus*, KU 197030; *posadae*, QCAZ 25090; *phenax*, KU 162264, 162266–67 (paratypes); *pluvialis*, KU 173225–27 (paratypes); *posadae* QCAZ 25090, 26022–23; *prasinus*, KU 169691–92 (paratypes); *siren*, KU 146611–23 (paratypes); *truebae*, KU 162269–80 (paratypes); *wileyi*, QCAZ 26029 (paratype).

Sachatamia: albomaculata, KU 65185, QCAZ 4325; *ilex*, ICN 10625–29, 10630, 10631–32, KU 116464, LACM 72910.

Teratohyla: cf. *amelie* MNCN 44212, MHNC 5646; *midas*, KU 123219; *pulverata*, QCAZ 32066, 32224; *spinosa*, KU 164668, 32935.

Rulyrana: flavopunctata, QCAZ 32265, KU 121046; *spiculata*, KU 162283 (paratype); *saxiscandens*, KU 211800–01 (paratypes); *tangarana*, KU 21777 (paratype).

Vitreorana: ametarsia, MCZ A96522 (holotype), ICN 50847; *antisthenesi*, MNHLS 17909, KU 167775; *castroviejoi* 13356 (holotype); *eurygnatha*, KU 93225; *gorzulai* MNHLS 11221 (holotype); *helenae* MNHLS 9431 (holotype); *lema* MHNLS 17267,17142 (topotypes); *papillahallica*, UTA 52240; *parvula* BM 88-2-7-3 (lectotype).

APPENDIX 2.2. Current and new generic taxonomy of Glassfrogs (Centrolenidae). The current taxonomy follows the classification proposed by Ruiz-Carranza & Lynch (1991a), with the addition of *Nymphargus* Cisneros-Heredia & McDiarmid 2007. In the new taxonomy, placement of species is based on molecular data. *Species for which no molecular data were available, but that have morphological and/or behavioral characteristics that allow a tentative generic placement. **Species that we consider *Incertae sedis* within Centrolenidae because molecular data are not available, and morphological and behavioral characters do not provide unambiguous evidence on their phylogenetic position, for these species, we suggest to maintain the generic names provided in the current taxonomy, adding quotation to denote their uncertain phylogenetic position and to differentiate them from monophyletic clades. Molecular data is necessary for establishing the exact phylogenetic relationships of species.

Species	Author	Original genus	Current taxonomy	New taxonomy
acanthidiocephala	Ruiz-Carranza & Lynch 1989	*Centrolenella*	*Centrolene acanthidiocephalum*	** *"Centrolene" acanthidiocephalum*
adenocheira	Harvey & Noonan 2005	*Cochranella*	*Cochranella adenocheira*	** *"Cochranella" adenocheira*
adiazeta	Ruiz-Carranza & Lynch 1991d	*Cochranella*	*Cochranella adiazeta*	*Rulyrana adiazeta*
albomaculata	Taylor 1949	*Centrolenella*	*Cochranella albomaculata*	*Sachatamia albomaculata*
altitudinalis	Rivero 1968	*Centrolenella*	*Centrolene altitudinale*	*Centrolene altitudinale*
amelie	Cisneros-Heredia & Meza-Ramos 2007	*Cochranella*	*Cochranella amelie*	*Teratohyla amelie*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
ametarsia	Flores 1987	*Centrolenella*	*Cochranella ametarsia*	** Vitreorana ametarsia*
andina	Rivero 1968	*Centrolenella*	*Centrolene andinum*	*Espadarana andina*
anomala	Lynch & Duellman 1973	*Centrolenella*	*Nymphargus anomalus*	** Nymphargus anomalus*
antioquiensis	Noble 1920	*Centrolenella*	*Centrolene antioquiense*	*Centrolene antioquiense*
antisthenesi	Goin 1963	*Centrolenella*	*Hyalinobatrachium antisthenesi*	*Vitreorana antisthenesi*
armata	Lynch & Ruiz-Carranza 1996	*Cochranella*	*Nymphargus armatus*	** Nymphargus armatus*
audax	Lynch & Duellman 1973	*Centrolenella*	*Centrolene audax*	*** "Centrolene" audax*
aureoguttata	Barrera-Rodríguez & Ruiz-Carranza 1989	*Centrolenella*	*Hyalinobatrachium aureoguttatum*	*Hyalinobatrachium aureoguttatum*
azulae	Flores & McDiarmid 1989	*Centrolenella*	*Centrolene azulae*	*** "Centrolene" azulae*
bacatum	Wild 1994	*Centrolene*	*Centrolene bacatum*	*Centrolene bacatum*
balionota	Duellman 1981	*Centrolenella*	*Cochranella balionota*	*** "Cochranella" balionota*
ballux	Duellman & Burrowes 1989	*Centrolenella*	*Centrolene ballux*	*** "Centrolene" ballux*
bejaranoi	Cannatella 1980	*Centrolenella*	*Nymphargus bejaranoi*	*Nymphargus bejaranoi*
bergeri	Cannatella 1980	*Centrolenella*	*Hyalinobatrachium bergeri*	*Hyalinobatrachium bergeri*
buckleyi	Boulenger 1882	*Hylella*	*Centrolene buckleyi*	*Centrolene buckleyi*
buckelyi venezuelensis	Rivero 1968	*Centrolenella*	*Centrolene venezuelense*	*Centrolene venezuelense*
buenaventura	Cisneros-Heredia & Yánez-Muñoz 2007	*Cochranella*	*Nymphargus buenaventura*	** Nymphargus buenaventura*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
callistommum	Guayasamin & Trueb 2007	*Centrolene*	*Centrolene callistommum*	*Espadarana callistomma*
cariticommata	Wild 1994	*Cochranella*	*Nymphargus cariticommatus*	* *Nymphargus cariticommatus*
castroviejoi	Ayarzagüena & Señaris 1996	*Cochranella*	*Cochranella castroviejoi*	*Vitreorana castroviejoi*
chami	Ruiz-Carranza & Lynch 1995b	*Cochranella*	*Nymphargus chami*	* *Nymphargus chami*
chancas	Duellman & Schulte 1993	*Cochranella*	*Nymphargus chancas*	* *Nymphargus chancas*
chirripoi	Taylor 1958	*Cochranella*	*Hyalinobatrachium chirripoi*	*Hyalinobatrachium chirripoi*
cochranae	Goin 1961	*Cochranella*	*Nymphargus cochranae*	*Nymphargus cochranae*
colymbiphyllum	Taylor 1949	*Centrolenella*	*Hyalinobatrachium colymbiphyllum*	*Hyalinobatrachium colymbiphyllum*
cristinae	Ruiz-Carranza & Lynch 1995b	*Cochranella*	*Nymphargus cristinae*	* *Nymphargus cristinae*
croceopodes	Duellman & Schulte 1993	*Cochranella*	*Cochranella croceopodes*	** *"Cochranella" croceopodes*
crurifasciatum	Myers & Donnelly 1997	*Hyalinobatrachium*	*Hyalinobatrachium crurifasciatum*	*Hyalinobatrachium crurifasciatum*
daidalea	Ruiz-Carranza & Lynch 1991c	*Cochranella*	*Cochranella daidalea*	*Centrolene daidaleum*
duidaeana	Ayarzagüena 1992	*Centrolenella*	*Cochranella duidaeana*	** *"Cochranella" duidaeana*
duranti	Rivero 1985	*Centrolenella*	*Hyalinobatrachium duranti*	*Hyalinobatrachium duranti*
durrellorum	Cisneros-Heredia 2007	*Centrolene*	*Centrolene durrellorum*	** *"Centrolene" durrellorum*
eccentricum	Myers & Donnelly 2001	*Hyalinobatrachium*	*Hyalinobatrachium eccentricum*	*Hyalinobatrachium eccentricum*
erminea	Torres-Gastello, Suárez-Segovia, & Cisneros-Heredia	*Cochranella*	*Cochranella erminea*	** *"Cochranella" erminea*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
esmeralda	Ruiz-Carranza & Lynch 1998	*Hyalinobatrachium*	*Hyalinobatrachium esmeralda*	* *Hyalinobatrachium esmeralda*
euhystrix	Cadle & McDiarmid 1990	*Centrolenella*	*Cochranella euhystrix*	** *"Cochranella" euhystrix*
euknemos	Savage & Starrett 1967	*Centrolenella*	*Cochranella euknemos*	*Cochranella euknemos*
eurygnatha	Lutz 1925	*Hyla*	*Hyalinobatrachium eurygnathum*	*Vitreorana eurygnatha*
fernandoi	Duellman & Schulte 1993	*Centrolene*	*Centrolene fernandoi*	** *"Centrolene" fernandoi*
flavopunctata	Lynch & Duellman 1973	*Centrolenella*	*Cochranella flavopunctata*	*Rulyrana flavopunctata*
fleischmanni	Boettger 1893	*Hylella*	*Hyalinobatrachium fleischmanni*	*Hyalinobatrachium fleischmanni*
fragilis	Rivero 1985	*Centrolenella*	*Hyalinobatrachium fragile*	*Hyalinobatrachium fragile*
garciae	Ruiz-Carranza & Lynch 1995a	*Cochranella*	*Nymphargus garciae*	*Nymphargus garciae*
geckoideum	Jiménez de la Espada 1872	*Centrolene*	*Centrolene geckoideum*	*Centrolene geckoideum*
geijskesi	Goin 1966	*Centrolenella*	*Cochranella geijskesi*	** *"Cochranella" geijskesi*
gemmata	Flores 1985	*Centrolenella*	*Centrolene gemmatum*	* *Centrolene gemmatum*
gorzulae	Ayarzagüena 1992	*Centrolenella*	*Centrolene gorzulai*	*Vitreorana gorzulai*
grandisonae	Cochran & Goin 1970	*Centrolenella*	*Centrolene grandisonae*	*Nymphargus grandisonae*
granulosa	Taylor 1949	*Centrolenella*	*Cochranella granulosa*	*Cochranella granulosa*
griffithsi	Goin 1961	*Cochranella*	*Nymphargus griffithsi*	*Nymphargus griffithsi*
guairarepanensis	Señaris 2001	*Hyalinobatrachium*	*Hyalinobatrachium guairarepanensis*	**Hyalinobatrachium guairarepanense*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
guanacarum	Ruiz-Carranza & Lynch 1995c	*Centrolene*	*Centrolene guanacarum*	*** "Centrolene" guanacarum*
helenae	Ayarzagüena 1992	*Centrolenella*	*Cochranella helenae*	*Vitreorana helenae*
heloderma	Duellman 1981	*Centrolenella*	*Centrolene heloderma*	** Centrolene heloderma*
hesperia	Cadle & McDiarmid 1990	*Centrolenella*	*Centrolene hesperium*	*Centrolene hesperium*
huilense	Ruiz-Carranza & Lynch 1995c	*Centrolene*	*Centrolene huilense*	*** "Centrolene" huilense*
hybrida	Ruiz-Carranza & Lynch 1991b	*Centrolene*	*Centrolene hybrida*	*Centrolene hybrida*
iaspidiensis	Ayarzagüena 1992	*Centrolenella*	*Hyalinobatrachium iaspidiense*	*Hyalinobatrachium iaspidiense*
ibama	Ruiz-Carranza & Lynch 1998	*Hyalinobatrachium*	*Hyalinobatrachium ibama*	*Hyalinobatrachium ibama*
ignioculus	Noonan & Bonett 2003	*Hyalinobatrachium*	*Hyalinobatrachium ignioculus*	*Hyalinobatrachium ignioculus*
ignota	Lynch 1990	*Centrolenella*	*Nymphargus ignotus*	** Nymphargus ignotus*
ilex	Savage 1967	*Centrolenella*	*Centrolene ilex*	*Sachatamia ilex*
laurae	Cisneros-Heredia & McDiarmid 2007	*Nymphargus*	*Nymphargus laurae*	** Nymphargus laurae*
lema	Duellman & Señaris 2003	*Centrolene*	*Centrolene lema*	*Vitreorana lema*
lemniscatum	Duellman & Schulte 1993	*Centrolene*	*Centrolene lemniscatum*	*** "Centrolene" lemniscatum*
lemur	Duellman & Schulte 1993	*Hyalinobatrachium*	*Hyalinobatrachium lemur*	** Hyalinobatrachium lemur*
litoralis	Ruiz-Carranza & Lynch 1996	*Centrolene*	*Centrolene litorale*	*Cochranella litoralis*
luminosa	Ruiz-Carranza & Lynch 1995b	*Cochranella*	*Nymphargus luminosa*	** Nymphargus luminosus*
luteopunctata	Ruiz-Carranza & Lynch 1996	*Cochranella*	*Nymphargus luteopunctatus*	** Nymphargus luteopunctatus*
lynchi	Duellman 1980	*Centrolenella*	*Centrolene lynchi*	** Centrolene lynchi*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
mache	Guayasamin & Bonaccorso 2004	*Cochranella*	*Cochranella mache*	*Cochranella mache*
mariae	Duellman & Toft 1979	*Centrolenella*	*Centrolene mariae*	* *Nymphargus mariae*
mariaelenae	Cisneros-Heredia & McDiarmid 2006	*Centrolene*	*Centrolene mariaelenae*	*Chimerella mariaelenae*
medemi	Cochran & Goin 1970	*Centrolenella*	*Centrolene medemi*	** *"Centrolene" medemi*
megacheira	Lynch & Duellman 1973	*Centrolenella*	*Nymphargus megacheirus*	*Nymphargus megacheirus*
megistra	Rivero 1985	*Centrolenella*	*Cochranella megista*	** *"Cochranella" megista*
midas	Lynch & Duellman 1973	*Centrolenella*	*Cochranella midas*	*Teratohyla midas*
mixomaculata	Guayasamin Lehr Rodriguez Aguilar 2006a	*Cochranella*	*Nymphargus mixomaculatus*	*Nymphargus mixomaculatus*
mondolfii	Señaris & Ayarzagüena 2001	*Hyalinobatrachium*	*Hyalinobatrachium mondolfii*	*Hyalinobatrachium mondolfii*
muelleri	Duellman & Schulte 1993	*Centrolene*	*Centrolene muelleri*	** *"Centrolene" muelleri*
munozorum	Lynch & Duellman 1973	*Centrolenella*	*Hyalinobatrachium munozorum*	*Hyalinobatrachium munozorum*
nephelophila	Ruiz-Carranza & Lynch 1991d	*Cochranella*	*Nymphargus nephelophila*	* *Nymphargus nephelophilus*
nola	Harvey 1996	*Cochranella*	*Cochranella nola*	*Cochranella nola*
notostictum	Ruiz-Carranza & Lynch 1991b	*Centrolene*	*Centrolene notostictum*	*Centrolene notostictum*
nouraguensis	Lescure & Marty 2000	*Hyalinobatrachium*	*Hyalinobatrachium nouraguensis*	*Hyalinobatrachium nouraguense*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
ocellata	Boulenger 1918	*Hylella*	*Cochranella ocellata*	* *Nymphargus ocellatus*
orejuela	Duellman & Burrowes 1989	*Centrolenella*	*Cochranella orejuela*	** *"Cochranella" orejuela*
oreonympha	Ruiz-Carranza & Lynch 1991d	*Cochranella*	*Nymphargus oreonympha*	* *Nymphargus oreonympha*
orientalis	Rivero 1968	*Centrolenella*	*Hyalinobatrachium orientale*	*Hyalinobatrachium orientale*
orocostalis	Rivero 1968	*Centrolenella*	*Hyalinobatrachium orocostalis*	*Hyalinobatrachium orocostale*
oyampiensis	Lescure 1975	*Cochranella*	*Cochranella oyampiensis*	*Vitreorana oyampiensis*
paezorum	Ruiz-Carranza Hernández-Camacho & Ardila-Robayo 1986	*Centrolene*	*Centrolene paezorum*	* *Centrolene paezorum*
pallida	Rivero 1985	*Centrolenella*	*Hyalinobatrachium pallidum*	*Hyalinobatrachium pallidum*
papillahallicum	Noonan & Harvey 2000	*Centrolene*	*Centrolene papillahallicum*	*Vitreorana papillahallica*
parvula	Boulenger 1895	*Hylella*	*Hyalinobatrachium parvulum*	* *Vitreorana parvula*
pellucida	Lynch & Duellman 1973	*Centrolenella*	*Hyalinobatrachium pellucidum*	*Hyalinobatrachium pellucidum*
peristicta	Lynch & Duellman 1973	*Centrolenella*	*Centrolene peristictum*	*Centrolene peristictum*
petersi	Goin 1961	*Cochranella*	*Hyalinobatrachium petersi*	**Hyalinobatrachium petersi*
petrophilum	Ruiz-Carranza & Lynch 1991b	*Centrolene*	*Centrolene petrophilum*	** *"Centrolene" petrophilum*
phenax	Cannatella & Duellman 1982	*Centrolenella*	*Nymphargus phenax*	* *Nymphargus phenax*
phryxa	Aguayo & Harvey 2006	*Cochranella*	*Cochranella phryxa*	* *Cochranella phryxa*
pipilata	Lynch & Duellman 1973	*Centrolenella*	*Centrolene pipilatum*	*Centrolene pipilatum*
pluvialis	Cannatella & Duellman 1982	*Centrolenella*	*Nymphargus pluvialis*	*Nymphargus pluvialis*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
posadae	Ruiz-Carranza & Lynch 1995a	*Cochranella*	*Nymphargus posadae*	*Nymphargus posadae*
prasina	Duellman 1981	*Centrolenella*	*Nymphargus prasinus*	* *Nymphargus prasinus*
prosoblepon	Boettger 1892	*Hyla*	*Centrolene prosoblepon*	*Espadarana prosoblepon*
pulverata	Peters 1873	*Hyla*	*Hyalinobatrachium pulveratum*	*Teratohyla pulverata*
punctulata	Ruiz-Carranza & Lynch 1995a	*Cochranella*	*Cochranella punctulata*	*Sachatamia punctulata*
puyoensis	Flores & McDiarmid 1989	*Centrolenella*	*Cochranella puyoensis*	*Nymphargus puyoensis*
quindianum	Ruiz-Carranza & Lynch 1995c	*Centrolene*	*Centrolene quindianum*	** *"Centrolene" quindianum*
ramirezi	Ruiz-Carranza & Lynch 1991c	*Cochranella*	*Cochranella ramirezi*	** *"Cochranella" ramirezi*
resplendens	Lynch & Duellman 1973	*Centrolenella*	*Cochranella resplendens*	* *Cochranella resplendens*
revocata	Rivero 1985	*Centrolenella*	*Cochranella revocata*	*Celsiella revocata*
ritae	Lutz 1952	*Centrolene*	*Cochranella ritae*	** *"Cochranella" ritae*
riveroi	Ayarzagüena 1992	*Centrolenella*	*Cochranella riveroi*	** *"Cochranella" riveroi*
robledoi	Ruiz-Carranza & Lynch 1995c	*Centrolene*	*Centrolene robledoi*	** *"Centrolene" robledoi*
rosada	Ruiz-Carranza & Lynch 1997	*Cochranella*	*Nymphargus rosada*	*Nymphargus rosadus*
ruedai	Ruiz-Carranza & Lynch 1998	*Hyalinobatrachium*	*Hyalinobatrachium ruedai*	**Hyalinobatrachium ruedai*
ruizi	Lynch 1993	*Cochranella*	*Nymphargus ruizi*	* *Nymphargus ruizi*
sanchezi	Ruiz-Carranza & Lynch 1991b	*Centrolene*	*Centrolene sanchezi*	* *Centrolene sanchezi*
savagei	Ruiz-Carranza & Lynch 1991c	*Cochranella*	*Cochranella savagei*	*Centrolene savagei*
saxiscandens	Duellman & Schulte 1993	*Cochranella*	*Cochranella saxiscandens*	* *Rulyrana saxiscandens*
scirtetes	Duellman & Burrowes 1989	*Centrolenella*	*Centrolene scirtetes*	* *Centrolene scirtetes*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
siren	Lynch & Duellman 1973	*Centrolenella*	*Nymphargus siren*	*Nymphargus siren*
solitaria	Ruiz-Carranza & Lynch 1991c	*Cochranella*	*Cochranella solitaria*	* *Centrolene solitaria*
spiculata	Duellman 1976	*Cochranella*	*Cochranella spiculata*	*Rulyrana spiculata*
spilota	Ruiz-Carranza & Lynch 1997	*Cochranella*	*Nymphargus spilotus*	* *Nymphargus spilotus*
spinosa	Taylor 1949	*Centrolenella*	*Cochranella spinosa*	*Teratohyla spinosa*
susatamai	Ruiz-Carranza & Lynch 1995a	*Cochranella*	*Cochranella susatamai*	*Rulyrana susatamai*
talamancae	Taylor 1952	*Cochranella*	*Hyalinobatrachium talamancae*	**Hyalinobatrachium talamancae*
tangarana	Duellman & Schulte 1993	*Cochranella*	*Cochranella tangarana*	* *Rulyrana tangarana*
tatayoi	Castroviejo-Fisher Ayarzagüena & Vilà 2007	*Hyalinobatrachium*	*Hyalinobatrachium tatayoi*	*Hyalinobatrachium tatayoi*
taylori	Goin 1968	*Centrolenella*	*Hyalinobatrachium taylori*	*Hyalinobatrachium taylori*
tayrona	Ruiz-Carranza & Lynch 1991b	*Centrolene*	*Centrolene tayrona*	*Ikakogi tayrona*
truebae	Duellman 1976	*Centrolenella*	*Nymphargus truebae*	* *Nymphargus truebae*
uranoscopa	Müller 1924	*Hyla*	*Hyalinobatrachium uranoscopum*	* *Vitreorana uranoscopa*
valerioi	Dunn 1931	*Centrolene*	*Hyalinobatrachium valerioi*	*Hyalinobatrachium valerioi*
vireovittata	Starrett & Savage 1973	*Centrolenella*	*Hyalinobatrachium vireovittatum*	*Hyalinobatrachium vireovittatum*
vozmedianoi	Ayarzagüena & Señaris 1996	*Cochranella*	*Cochranella vozmedianoi*	*Celsiella vozmedianoi*

APPENDIX 2.2. Continued.

Species	Author	Original genus	Current taxonomy	New taxonomy
wileyi	Guayasamin, Bustamante, Almeida-Reinoso & Funk 2006b	*Cochranella*	*Nymphargus wileyi*	*Nymphargus wileyi*
xanthocheridia	Ruiz-Carranza & Lynch 1995b	*Cochranella*	*Cochranella xanthocheridia*	** *"Cochranella" xanthocheridia*

APPENDIX 3.1. Description of morphological and behavioral character states in Glassfrogs.

1. Eggs' deposition site: (0) Eggs deposited on the uppersides of leaves; (1) Eggs deposited on the undersides of leaves.
2. Humeral spines in adult males: (0) Humeral spines present; (1) Humeral spines absent.
3. Ventral transparency: (0) Ventral parietal peritoneum white anteriorly and transparent posteriorly; (1) Ventral parietal peritoneum completely transparent.
4. Pericardium: (0) Heart covered by white peritoneum; (1) Heart covered by transparent peritoneum.
5. Hepatic peritoneum: (0) Liver covered by transparent hepatic peritoneum; (1) Liver covered by white hepatic peritoneum.
6. Gastrointestinal peritoneum: (0) Digestive tract covered by opaque hepatic peritoneum; (1) Digestive tract covered by white hepatic peritoneum.

APPENDIX 3.2**.** References and specimens examined for the morphological dataset. Museum abbreviations are as in Frost (2007). Cleared-and-stained = C&S.

Allophryne ruthveni: KU 167756 (C&S), 166731 (C&S), 166713–16. *Nymphargus bejaranoi:* KU 182370–71. *Nymphargus cochranae:* KU 121033–35, 123218 (C&S; Fig. 3.4), QCAZ 22196–97, 31113, 31340–41. *Nymphargus grandisonae:* KU 164674 (C&S), 164684 (C&S), KU 164686–90. *Nymphargus griffithsi:* KU 164520–25, 288991–92 (C&S), 118028–29 (C&S), 166322–23 (C&S), 166325–27 (C&S), 118148 (C&S). *Nymphargus megacheirus:* KU 143271 (C&S; Fig. 3.4), 143246–50, 166329. *Nymphargus megacheirus:* MHNSM 18653, 18632–33. *Nymphargus pluvialis:* KU 173488 (C&S; Fig. 3.4), 173225–27. *Nymphargus posadae:* ICN 7447–50. *Nymphargus rosadus:* ICN 34762, 34764, 34770. *Nymphargus siren:* KU 146611–13, 178203–04 (C&S), 166333 (C&S), QCAZ 30977–78. *Nymphargus wileyi:* QCAZ 26028, 26024–25, 26029 (C&S). *Celsiella revocata:* Señaris and Ayarzagüena (2005). *Celsiella vozmedianoi:* MHNLS 16427, 16430, Señaris and Ayarzagüena (2005). *Centrolene altitudinale:* Señaris and Ayarzagüena (2005). *Centrolene andinum:* MHNLS 16484–92. *Centrolene bacatum:* KU 170116 (C&S; Fig. 3.4), QCAZ 22386 (C&S), KU 202807–12. *Centrolene daidaleum:* ICN 8462–63, 14912, 14915–16 (C&S). *Centrolene hybrida:* ICN 9626, 17896, 10201 (C&S). *Centrolene peristictum:* KU 118052, 121053, 178150 (C&S), QCAZ 16316, 22312, 22314. *Centrolene pipilatum:* KU 143286 (C&S; Fig. 3.4), 143279–82. *Centrolene buckleyi:* KU 178042 (C&S), 189594 (C&S), 166321 (C&S), 202770–83. *Centrolene geckoideum:* ICN 5598 (C&S), KU 178016, 164492. *Centrolene gorzulai:* MHNLS 16036. *Centrolene grandisonae:* KU 164686–90, 164674 (C&S),

164684 (C&S). *Centrolene savagei:* ICN 9767, 24927, 29462. *Centrolene venezuelense:* Señaris and Ayarzagüena (2005). *Chimerella mariaelenae:* QCAZ 21252, 22363, 31729. *Spinabrachium callistommum:* QCAZ 28557 (C&S), 28803, 28555–58. *Spinabrachium prosoblepon*: KU 32376–80, 65178 (C&S), 178163 (C&S), 291165–70, *Sachatamia ilex:* ICN 10630 (C&S), 16687–88, KU 116464, LACM 72910 (Fig 3.1; redrawn from Hayes and Starrett, 1980). *Sachatamia punctulata:* ICN 34745, 34753. *Sachatamia albomaculata:* KU 65185 (C&S; Fig. 3.4), 80482–83, 108903. *Cochranella litoralis:* ICN 13821, QCAZ 27693 (Fig. 3.4). *Cochranella euknemos:* LACM 26764, KU 77534 (C&S; Fig. 3.4), 116436–38. *Cochranella granulosa:* KU 85474, UCR 16180, 16862. *Cochranella mache:* KU 291176, QCAZ 22412–13, 27747, 31327. *Cochranella nola:* Harvey (1996). *Hyalinobatrachium aureoguttatum:* ICN 17266, 17256, 17507, 17529 (C&S), 17261 (C&S), 17535 (C&S), QCAZ 4323, 6302, 6441–42, 27429, 6303 (C&S). *Hyalinobatrachium bergeri:* KU 162256 (C&S), 162258 (C&S), 182364–68. *Hyalinobatrachium cardiacalyptum:* USNM 538586. *Hyalinobatrachium chirripoi:* KU 36868 (C&S), 36862–64, 36866–67, ICN 39129–30. *Hyalinobatrachium colymbiphyllum:* ICN 30888, 19563, 19686 (C&S). *Hyalinobatrachium crurifasciatum:* MHNLS 16475, 16477. *Hyalinobatrachium duranti:* MHNLS 16493, 16498–99. *Hyalinobatrachium eccentricum:* Myers and Donnelly (2001). *Hyalinobatrachium fleischmanni:* KU 32960–69 (C&S), 68639–40. *Hyalinobatrachium fragile:* Señaris and Ayarzagüena (2005). *Hyalinobatrachium iaspidiense:* Señaris and Ayarzagüena (2005). *Hyalinobatrachium ibama:* ICN 10215–20. *Hyalinobatrachium ignioculus:* UTA A-51657, 51660. *Hyalinobatrachium lemur:* KU 211768–69. *Hyalinobatrachium mondolfii:* Señaris and Ayarzagüena (2005). *Hyalinobatrachium munozorum:* KU 155497 (C&S),

105251, 150620. *Hyalinobatrachium nouraguense:* Lescure and Marty (2000). *Hyalinobatrachium orientale:* KU 167369 (C&S), MHNLS 16443 (C&S), 16444–45, 16449. *Hyalinobatrachium pallidum:* Señaris and Ayarzagüena (2005). *Hyalinobatrachium pellucidum:* KU 143298. *Hyalinobatrachium taylori:* Señaris and Ayarzagüena (2005). *Hyalinobatrachium valerioi:* KU 178091 (C&S; Fig. 3.4), 104328 (C&S), ICN 38570–71. *Hyalinobatrachium vireovittatum:* CH 5330, 5532. *Ruizolynchus adiazetus:* ICN 17922–23, 4719 (C&S), 3554 (C&S). *Ruizolynchus flavopunctatus:* KU 121044,121049, 123224 (C&S), QCAZ 22360. *Ruizolynchus puyoensis:* MCZ 91187. *Ruizolynchus susatamai:* ICN 18641. *Ruizolynchus spiculatus:* KU 162283, 162283. *Vitreorana antisthenesi:* KU 133470, 133473–74, 133468 (C&S), 167338 (C&S), MHNLS 17909. *Vitreorana castroviejoi:* MHNLS 16446,16452,16429. *Vitreorana euryghnatha:* KU 93225 (C&S), 93225 (C&S; Fig. 3.4), 93220–23. *Vitreorana helenae:* MHNLS 16074. *Vitreorana lema:* KU 181128. *Vitreorana papillahallica:* KU 289208, UTA A-52239–40. *Teratohyla midas:* KU 123219, 107026, 125334, 146625, QCAZ 19316, 28286. *Teratohyla pulverata:* CH 5122, QCAZ 32224, 32066. *Teratohyla spinosa:* KU 32935 (C&S), ICN 19443, 16694.

APPENDIX 3.3. Randomly excluded species in the Reduced dataset.

Centrolene bacatum, C. buckleyi, C. grandisonae, C. peristictum, C. geckoideum, C. notostictum, C. savagei, C. venezuelense, Cochranella euknemos, , C. litoralis, C. mache, C. nola, Espadarana andina, E. sp.*, Hyalinobatrachium aureoguttatum, H. chirripoi, H. duranti, H. iaspidiense, H. fragile, H.* aff. *mondolfii, H.* cf. *pallidum, H. bergeri, H. pellucidum, H. ignioculus, H. nouraguensis, H.* cf. *munozorum, Nymphargus bejaranoi, N. cochranae, N.* cf. *cochranae, N. garciae, N. pluvialis, N. posadae, N. vozmedianoi, N. wileyi, Rulyrana adiazeta, R. flavopunctata, R. spiculata, R.* aff. *spiculata, R. susatamai, Sachatamia albomaculata, S. punctulata, Teratohyla* aff. *amelie, T. spinosa, Vitreorana antisthenesi, V. gorzulai, V. papillahallica.*

APPENDIX 3.4. Character state distribution in centrolenid species.

Species	Humeral spine	Egg's deposition site	Ventral transparency	Pericardial peritoneum	Hepatic peritoneum	Gastrointestinal peritoneum
Celsiella revocata	0	0/1	0	0	0	0
Celsiella vozmedianoi	0	0	0	0	0	0
Centrolene altitudinale	1	0	0	0	0	0
Centrolene antioquiense	1	0	0	0	0	1
Centrolene bacatum	1	0	0	0	0	0
Centrolene buckleyi	1	0	0	0	0	0
Centrolene daidaleum	0	?	0	0	0	1
Centrolene geckoideum	1	0	0	0	0	0
Centrolene hesperium	1	0	0	0	0	0
Centrolene hybrida	1	?	0	0	0	0
Centrolene notostictum	1	1	0	0	0	0
Centrolene peristictum	1	1	0	0	0	1
Centrolene pipilatum	1	?	0	0	0	1
Centrolene savagei	1	0	0	0	0	1
Centrolene venezuelense	1	0	0	0	0	0
Chimerella mariaelenae	1	?	1	0	1	1
Cochranella euknemos	0	0	0	0	0	1
Cochranella granulosa	0	0	0	0	0	1
Cochranella litoralis	1	?	0	0	0	1
Cochranella mache	0	0	0	0	0	1

APPENDIX 3.4. Continued.

Species	Humeral spine	Egg's deposition site	Ventral transparency	Pericardial peritoneum	Hepatic peritoneum	Gastrointestinal peritoneum
Cochranella nola	0	?	0	0	0	0
Cochranella sp	0	?	0	0	0	0
Espadarana andina	1	0	0	0	0	0
Espadarana callistomma	1	0	0	0	0	0
Espadarana prosoblepon	1	0	0	0	0	0
Espadarana sp	1	0	0	0	0	0
Hyalinobatrachium aureoguttatum	0	1	1	0/1	1	1
Hyalinobatrachium bergeri	0	1	1	0/1	1	1
Hyalinobatrachium chirripoi	0	1	1	1	1	1
Hyalinobatrachium colymbiphyllum	0	1	1	1	1	1
Hyalinobatrachium crurifasciatum	0	1	1	0/1	1	1
Hyalinobatrachium duranti	0	1	1	0	1	1
Hyalinobatrachium eccentricum	0	1	1	0/1	1	1
Hyalinobatrachium fleischmanni	0	1	1	0	1	1
Hyalinobatrachium fragile	0	1	1	1	1	1
Hyalinobatrachium iaspidiense	0	1	1	1	1	1
Hyalinobatrachium aff. *iaspidiense*	0	1	1	1	1	1
Hyalinobatrachium ibama	0	1	1	0	1	1
Hyalinobatrachium cf. *ignioculus*	0	1	1	0	1	1
Hyalinobatrachium mondolfii	0	1	1	0	1	1

APPENDIX 3.4. Continued.

Species	Humeral spine	Egg's deposition site	Ventral transparency	Pericardial peritoneum	Hepatic peritoneum	Gastrointestinal peritoneum
Hyalinobatrachium aff. *mondolfii*	0	1	1	0	1	1
Hyalinobatrachium cf. *munozorum*	0	1	1	0	1	1
Hyalinobatrachium nouraguensis	0	1	1	1	1	1
Hyalinobatrachium orientale	0	1	1	1	1	1
Hyalinobatrachium orocostale	0	1	1	1	1	1
Hyalinobatrachium cf. *pallidum*	0	1	1	1	1	1
Hyalinobatrachium cf. *pellucidum*	0	?	1	1	1	1
Hyalinobatrachium sp	0	1	1	1	1	1
Hyalinobatrachium tatayoi	0	1	1	0	1	1
Hyalinobatrachium taylori	0	1	1	0/1	1	1
Hyalinobatrachium valerioi	0	1	1	0/1	1	1
Hyalinobatrachium vireovittatum	0	1	1	1	1	1
Ikakogi tayrona	1	0/1	0	0	0	0
Nymphargus bejaranoi	0	0	0	0	0	0
Nymphargus cochranae	0	0	0	0	0	0
Nymphargus garciae	0	0	0	0	0	0
Nymphargus grandisonae	1	0	0	0	0	0
Nymphargus griffithsi	0/1	0	0	0	0	0
Nymphargus megacheirus	0	0	0	0	0	0
Nymphargus mixomaculatus	0	0	0	0	0	0

APPENDIX 3.4. Continued.

Species	Humeral spine	Egg's deposition site	Ventral transparency	Pericardial peritoneum	Hepatic peritoneum	Gastrointestinal peritoneum
Nymphargus pluvialis	0	0	0	0	0	0
Nymphargus posadae	0	?	0	0	0	0
Nymphargus puyoensis	0	?	0	0	0	0
Nymphargus rosadus	0	0	0	0	0	0
Nymphargus siren	0	0	0	0	0	0
Nymphargus wileyi	0	0	0	0	0	0
Rulyrana adiazeta	0	0	0	0	0	0
Rulyrana flavopunctata	0	0	0	0	0	0
Rulyrana spiculata	0	?	0	0	0	0
Rulyrana susatamai	0	?	0	0	0	0
Sachatamia albomaculata	0	0/1	0	0	0	0
Sachatamia ilex	1	0	0	0	0	0
Sachatamia punctulata	0	?	0	0	0	0
Teratohyla aff. *amelie*	0	?	1	0	1	1
Teratohyla midas	0	0	0	0	0	1
Teratohyla pulverata	0	0	1	0	1	1
Teratohyla spinosa	0	1	0	0	0	0
Vitreorana antisthenesi	0	0	1	0	1	1
Vitreorana castroviejoi	0	0	0	0	1	1
Vitreorana eurygnatha	0	0/1	1	0	1	0

APPENDIX 3.4. Continued.

Species	Humeral spine	Egg's deposition site	Ventral transparency	Pericardial peritoneum	Hepatic peritoneum	Gastrointestinal peritoneum
Vitreorana gorzulai	1	?	1	0	1	1
Vitreorana helenae	0	?	0	0	1	1
Vitreorana lema	1	0	1	0	1	1
Vitreorana papillahallica	1	?	1	0	1	1
Vitreorana oyampiensis	0	?	0	0	0	1

FIGURES

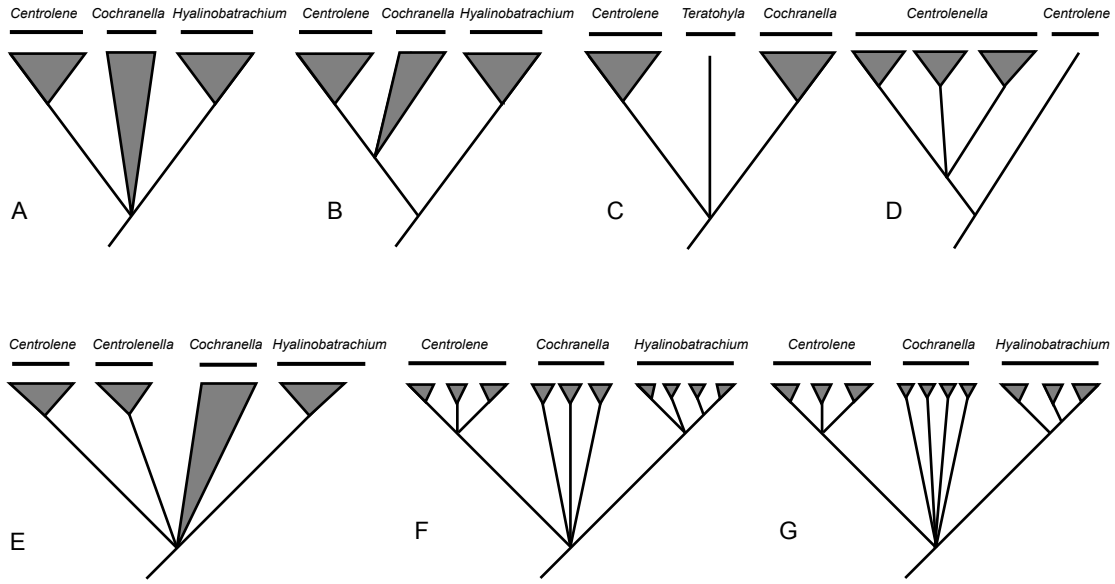

Fig. 1.1. Previous taxonomic hypotheses for centrolenid frogs. (A) Generic arrangement by Ruiz-Carranza and Lynch (1991). (B) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991), as modified by Bolívar et al. (1999). (C) Taxonomy sensu Taylor (1949, 1951). (D) Taxonomy sensu Savage (1967). (E) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991), as modified by Savage (2002). (F) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991, 1995, 1998). (G) Hypothesis of relationships sensu Ruiz-Carranza and Lynch (1991, 1995, 1998), as modified by Duellman and Señaris (2003), Señaris and Ayarzagüena (2005), and Cisneros-Heredia and McDiarmid (2006a, 2006b, 2007). In Fig. 1.1G, one the species groups within *Cochranella* corresponds to the genus *Nymphargus* sensu Cisneros-Heredia and McDiarmid (2007).

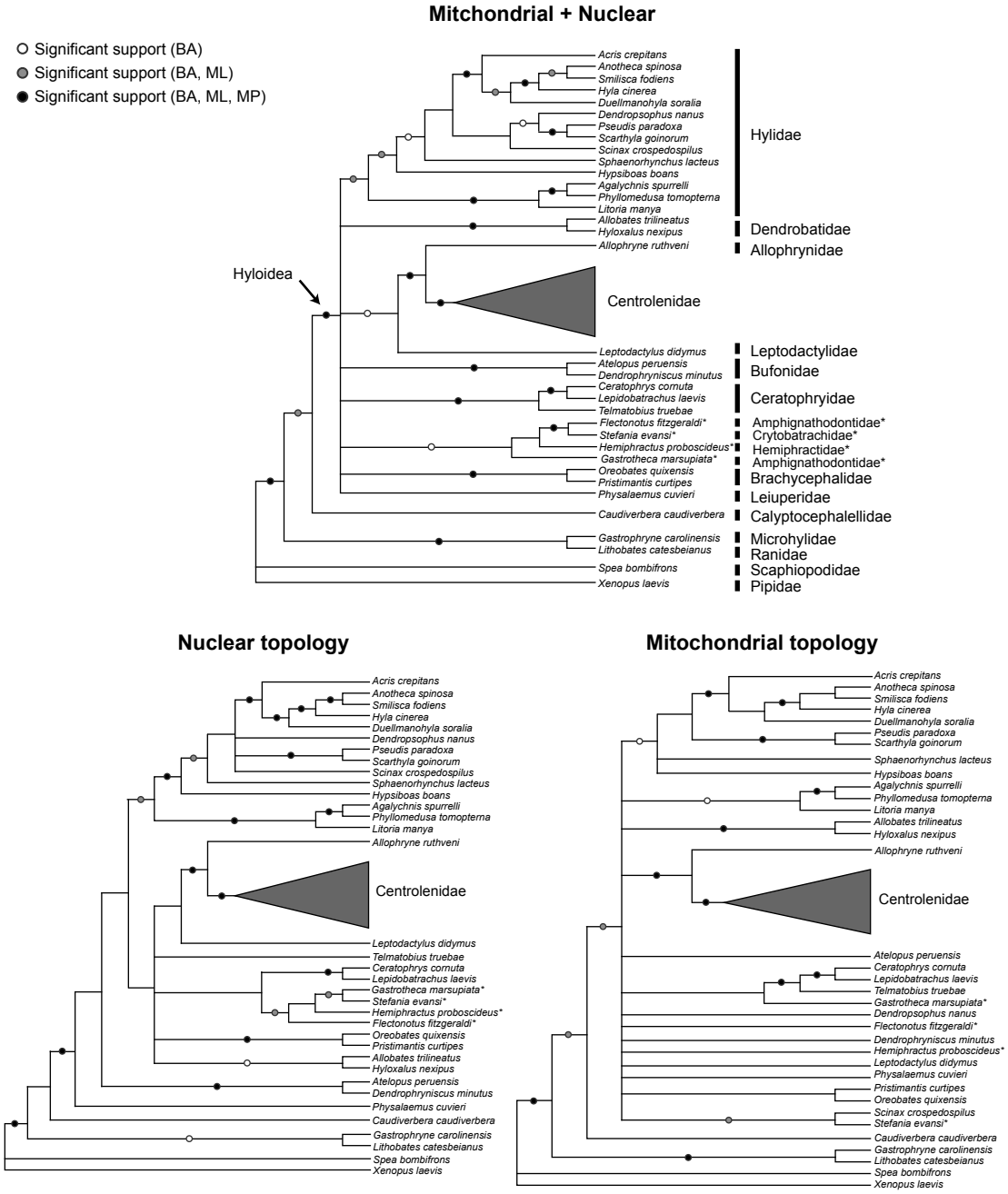

Fig. 1.2. Schematic tree summarizing relationships between Centrolenidae and other anurans. Note that the *Allophryne* + Centrolenidae clade is recovered consistently. Also, note that the topologies inferred from the nuclear and complete datasets support the monophyly of marsupial frogs (contra Frost et al., 2006). Circles indicate significant support values for clades recovered by Bayesian (posterior probability ≥ 0.95), Likelihood (bootstrap ≥ 70%), and Parsimony (bootstrap ≥ 70%) analyses.

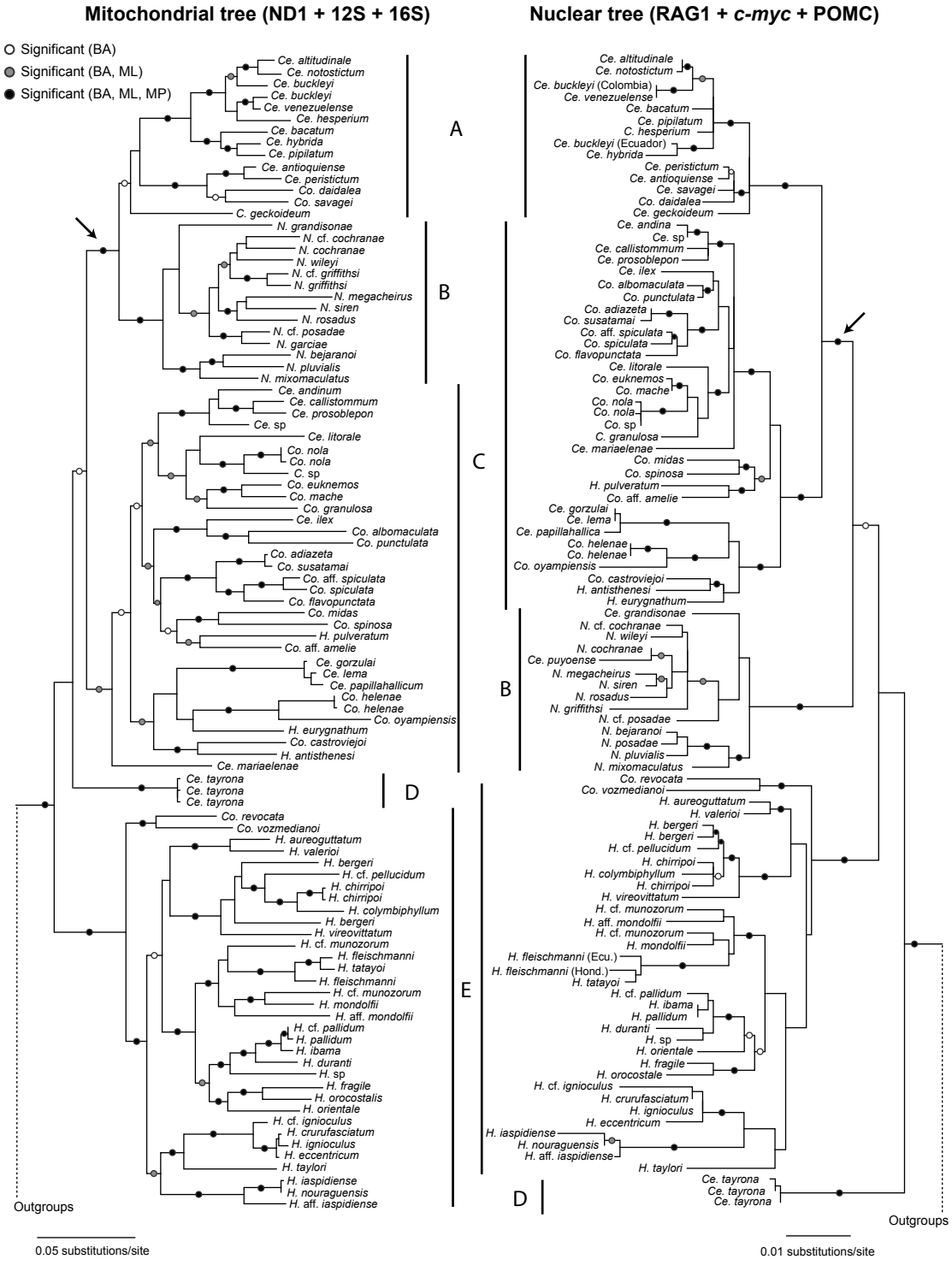

Fig.1.3. Phylogeny of Glassfrogs inferred from mitochondrial genes (12S, 16S, ND1; ln L = –74810.404) and nuclear genes (*c-myc* exon 2, Rag1, POMC; ln L = –17244.2). The tree was obtained using RAxML. Circles indicate significant support values.

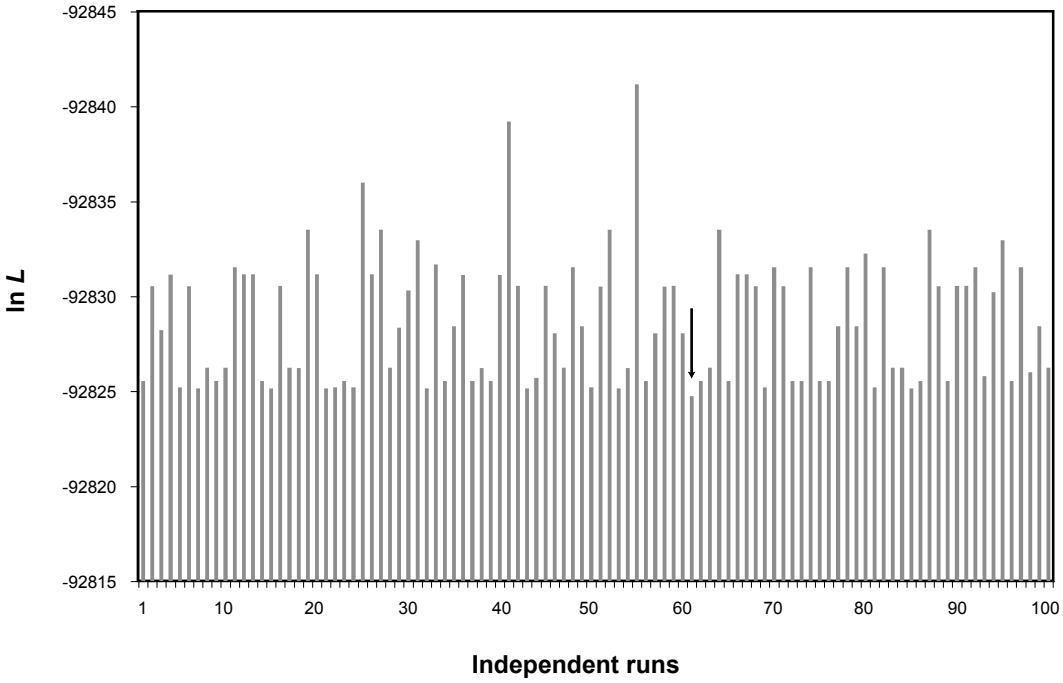

Fig. 1.4. Distribution of likelihood values inferred from the complete dataset using the program RAxML. The arrow indicates the run with the best likelihood (-92824.8).

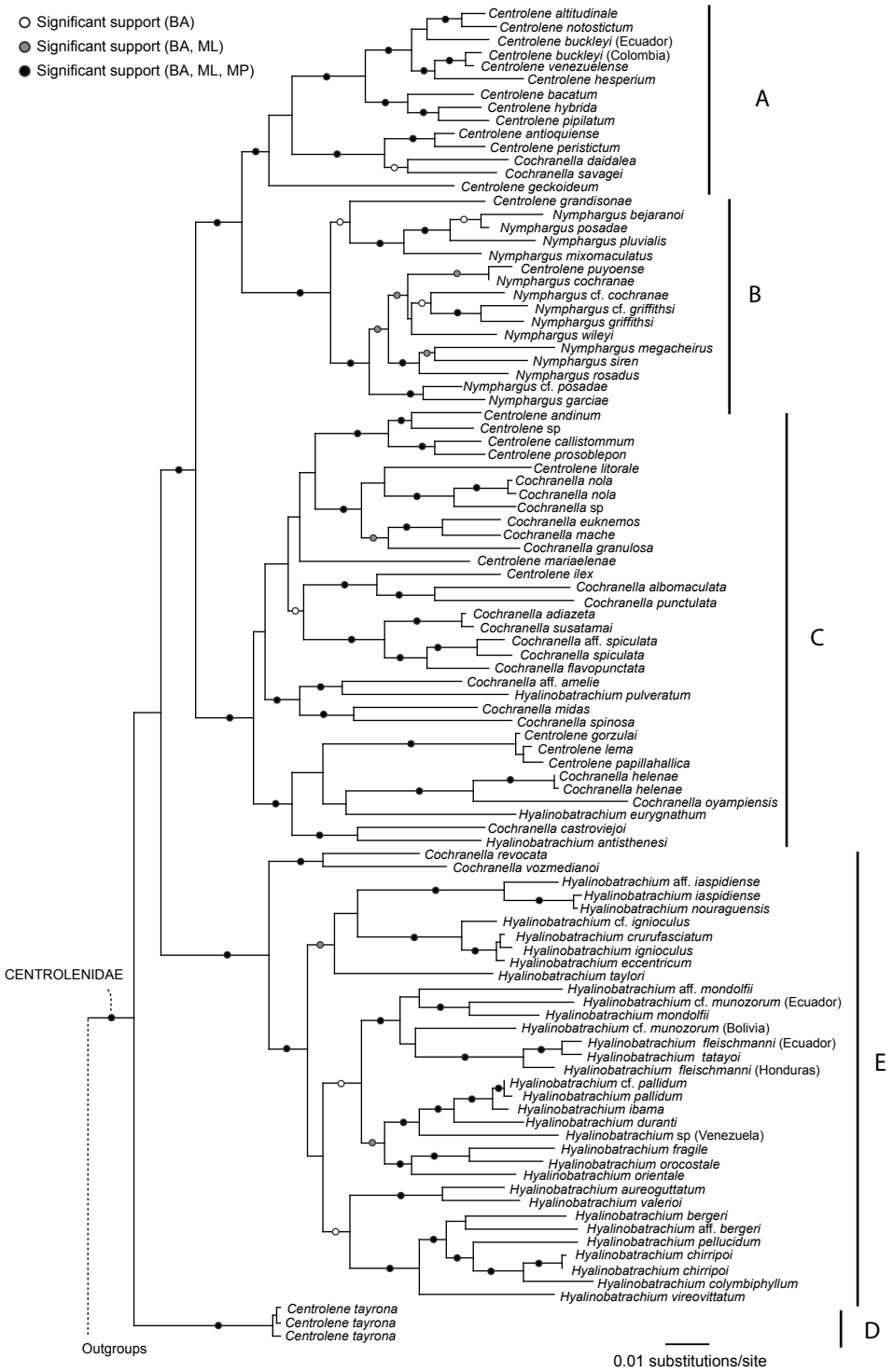

Fig. 1.5. Phylogeny of Glassfrogs inferred from the complete dataset using RAxML (ln *L* = -92824.772). Circles indicate significant support values for clades recovered by Bayesian (posterior probability ≥ 0.95), maximum likelihood (bootstrap ≥ 70%), and maximum parsimony (bootstrap ≥ 70%) analyses.

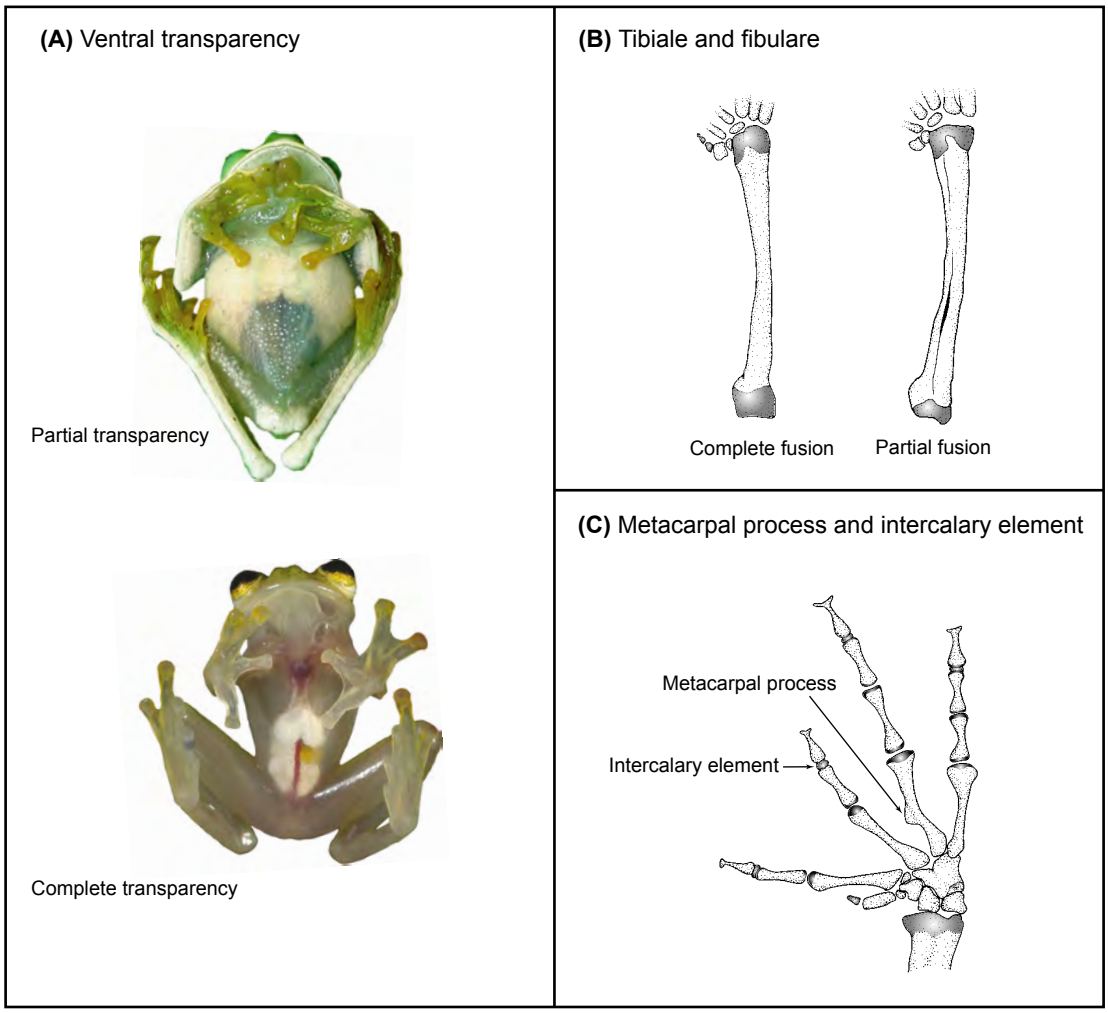

Fig. 2.1. Synapomorphies of Centrolenidae. **(A)** Partial (*Nymphargus posadae,* QCAZ 25090) and complete ventral transparency (*Hyalinobatrachium aureoguttatum,* QCAZ 32070). **(B)** Partial (*N. wileyi,* QCAZ 26029) and complete fusion between tibiale and fibulare (*H. munozorum,* KU 155497). **(C)** Medial process on Metacarpal III and intercalary element (*Teratohyla spinosa,* KU 32935). The presence of T or Y-shaped terminal phalanges is a synapomorphy of Centrolenia (Allophrynidae + Centrolenidae). Photos in (A) by M. Bustamante.

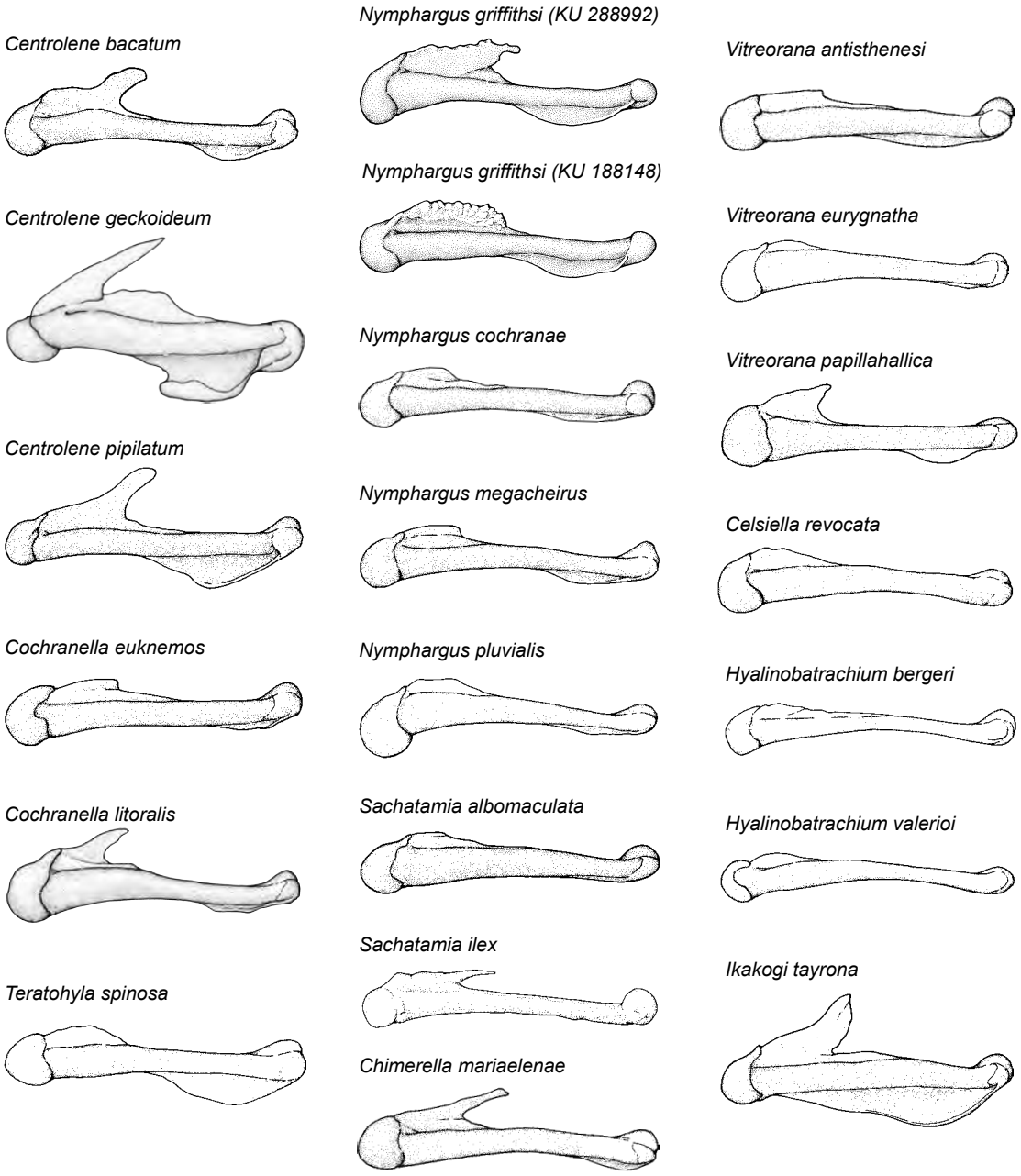

Fig. 2.10. Crista ventralis and humeral spines in centrolenid adult males. *Centrolene bacatum,* KU 170116; *C. geckoideum,* ICN 5598; *C. pipilatum,* KU 143286; *Cochranella euknemos,* KU 77534; *Cochranella litoralis,* QCAZ 27693; *Teratohyla spinosa,* KU 32935; *Nymphargus griffithsi,* KU 288992, 188148; *N. cochranae,* KU 123218; *N. megacheirus,* KU 143271; *N. pluvialis,* KU 173488; *Sachatamia albomaculata,* KU 65185; *S. ilex,* LACM 72910; *Chimerella mariaelenae,* QCAZ 21252; *Vitreorana antisthenesi,* KU 167775; *V. eurygnatha,* KU 93225; *V. papillahallica,* UTA 52240; *Celsiella revocata,* MHNLS 13352; *Hyalinobatrachium bergeri,* KU 162256; *H. valerioi,* KU 178091; *Ikakogi tayrona,* KU 169754.

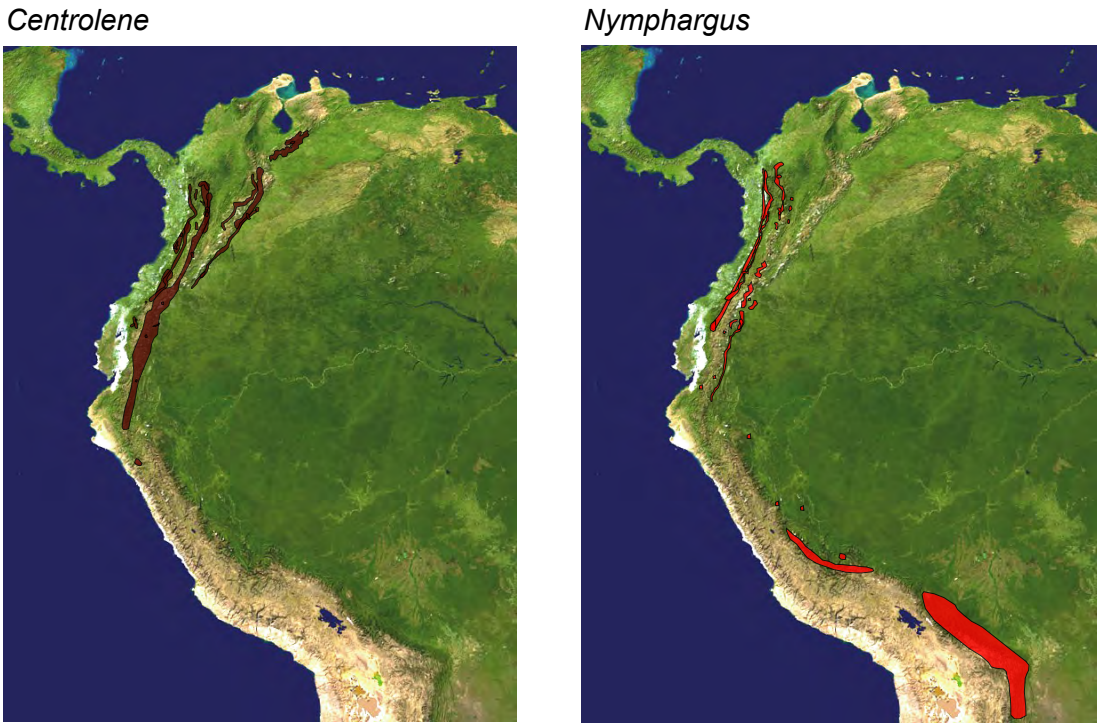

Fig. 2.11. Distribution of *Centrolene* and *Nymphargus*.

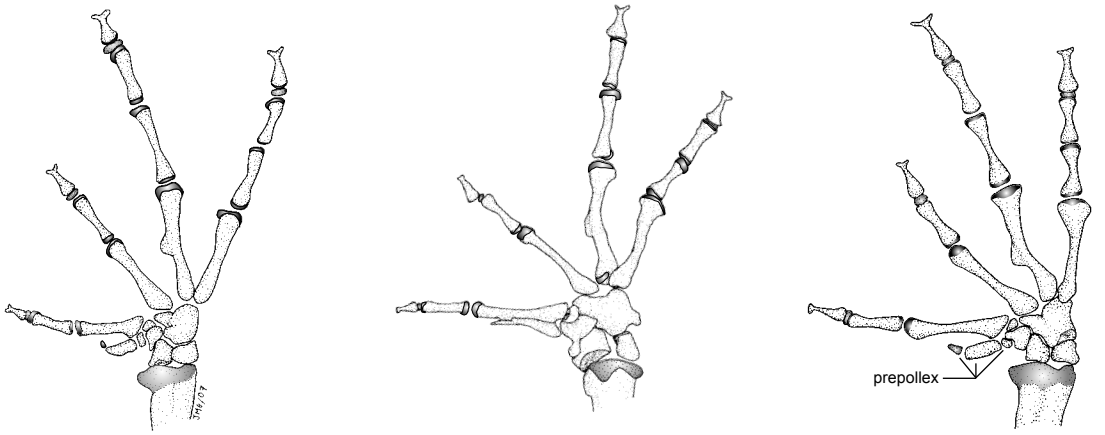

Fig. 2.12. Size of prepollex relative to Metacarpal I (dorsal view). Also, note relative length of Fingers I and II. Species illustrated: *Centrolene hesperium,* FMNH 232502; *Teratohyla spinsa,* KU 32935; *Hyalinobatrachium munozorum,* KU 155497.

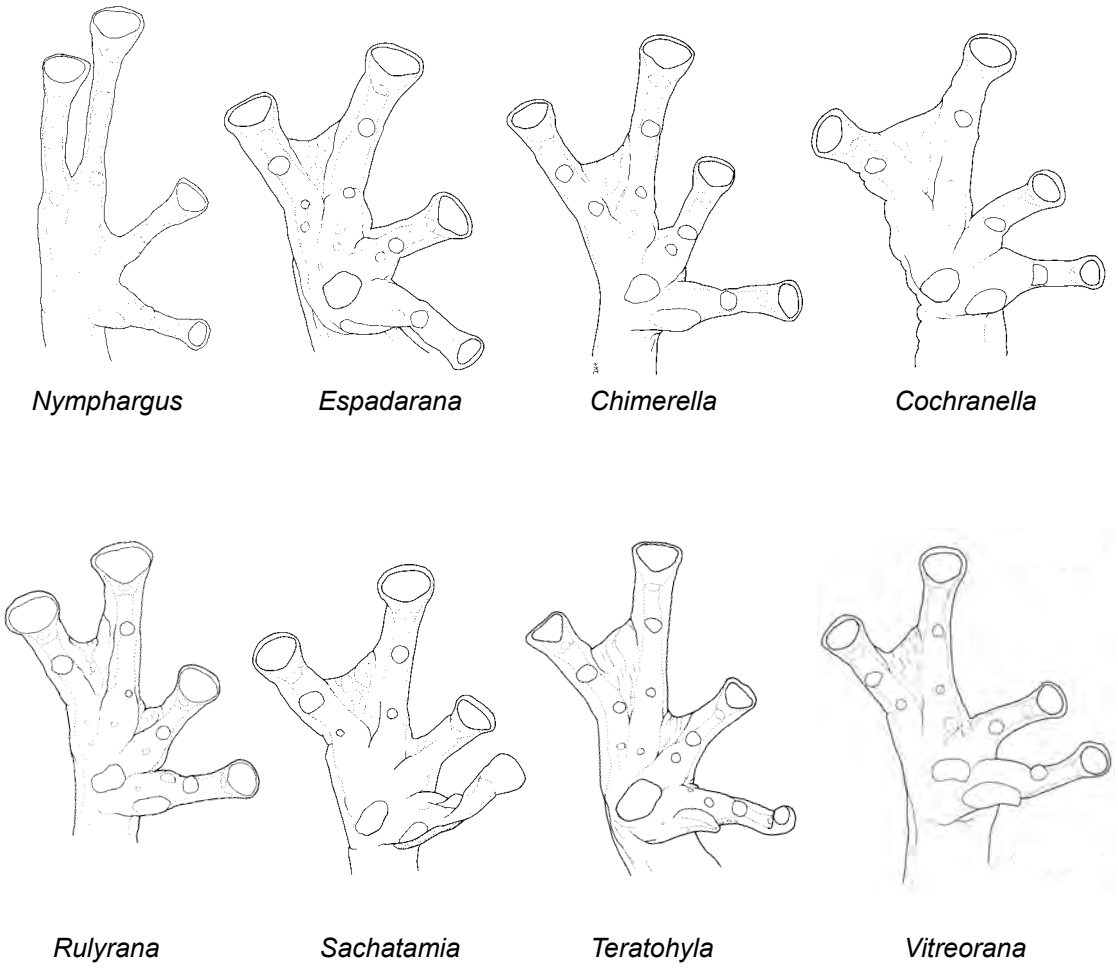

Fig. 2.13. Differences in hand webbing (Fingers III and IV) between *Nymphargus* and genera within Clade C. The following species are illustrated: *Nymphargus posadae,* QCAZ 25090; *Espadarana prosoblepon,* KU 132462; *Chimerella mariaelenae,* QCAZ 22363; *Cochranella resplendens,* KU 118053; *Rulyrana flavopunctata,* KU 121046; *Sachatamia albomaculata,* QCAZ 4325; *Teratohyla spinosa,* KU 164668; *Vitreorana ametarsia,* ICN 50847.

Fig. 2.14. Distribution of *Celsiella*, *Chimerella*, and *Ikakogi*.

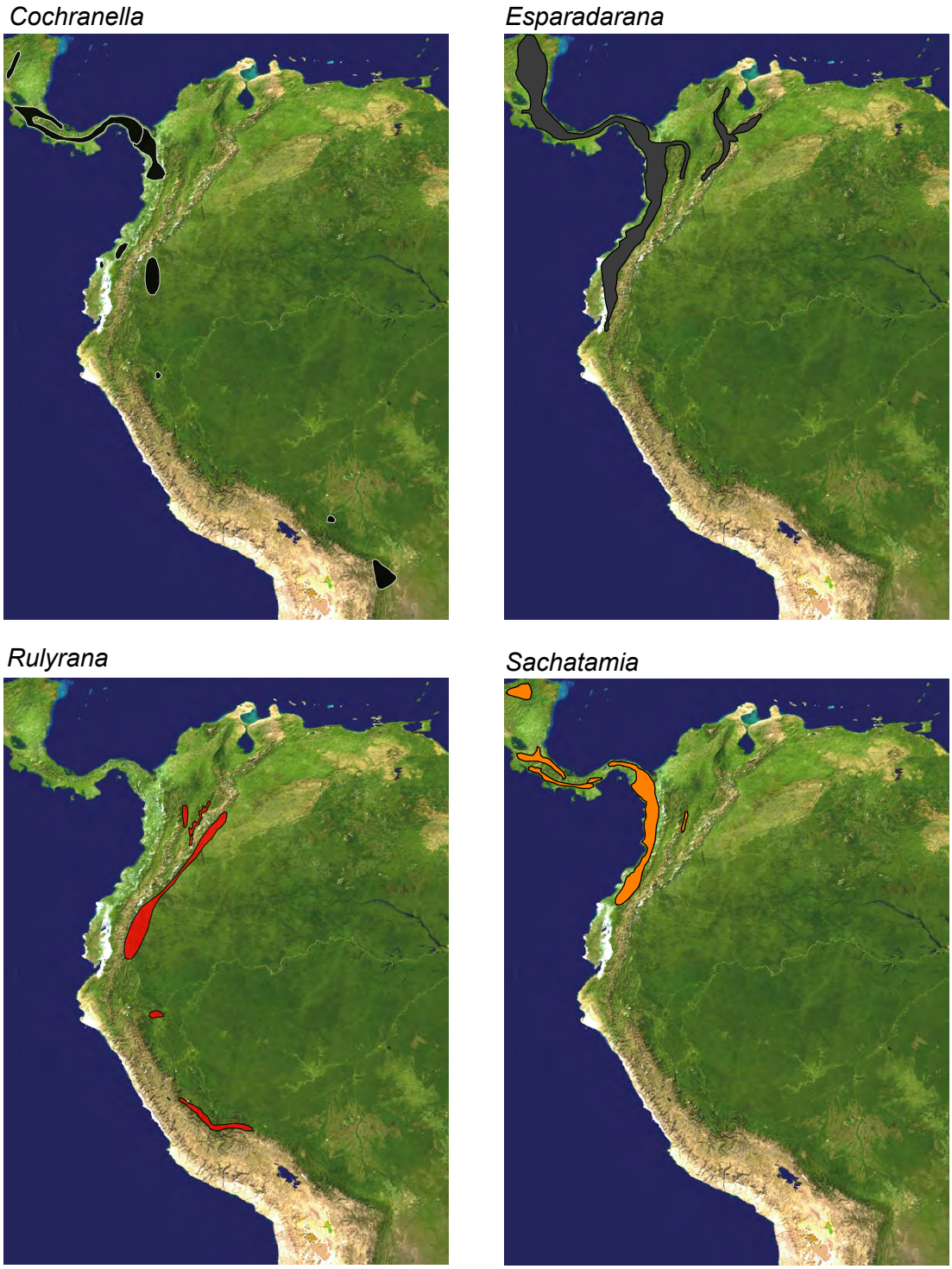

Fig. 2.15. Distribution of *Cochranella, Espadarana, Rulyrana,* and *Sachatamia.*

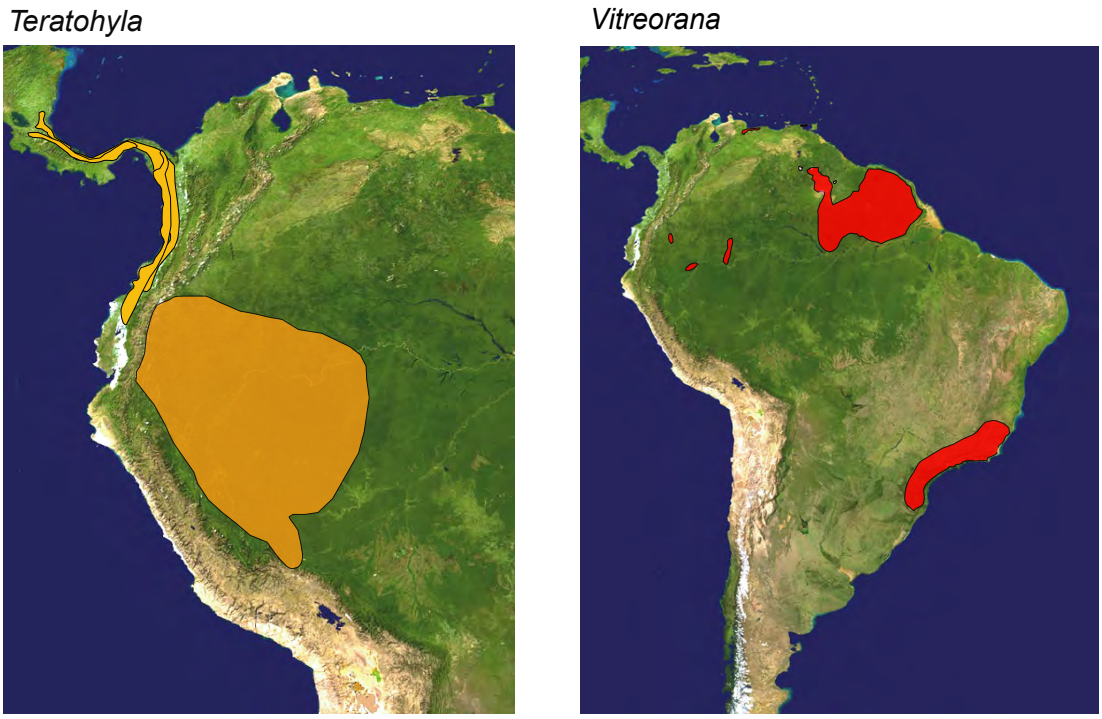

Fig. 2.16. Distribution of *Teratohyla* and *Vitreorana*.

Fig. 2.17. Distribution of Hyalinobatrachinae and *Hyalinobatrachium*.

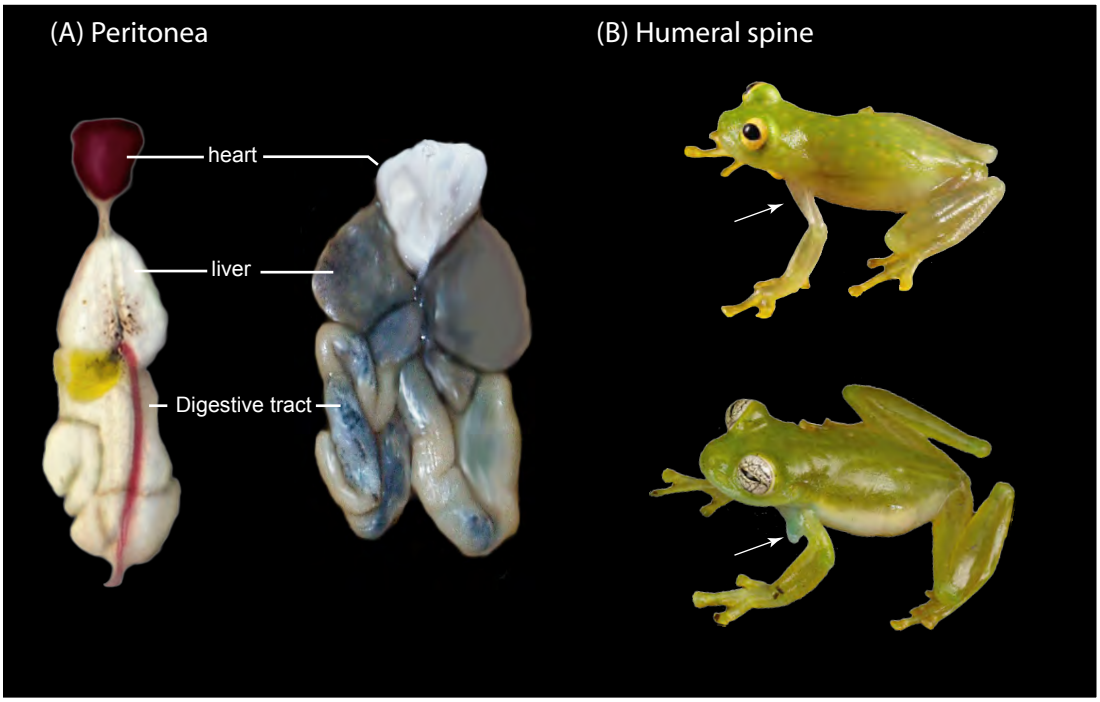

Fig. 2.2. Taxonomically relevant characters is Glassfrogs. **(A)** Transparent pericardium, white hepatic and visceral peritonea (left; *Hyalinobatrachium aureoguttatum*); white pericardium, translucent hepatic and visceral peritonea (right; *Centrolene buckleyi*). **(B)** Absence of humeral spine (top; *H. fleischmanni*); presence of humeral spine (bottom; *Espadarana callistomma*).

Fig. 2.3. Derived fighting behavior between males of *Espadarana andina*. An amplexus-like fighting behavior (not shown) is considered primitive (Bolívar et al., 1999).

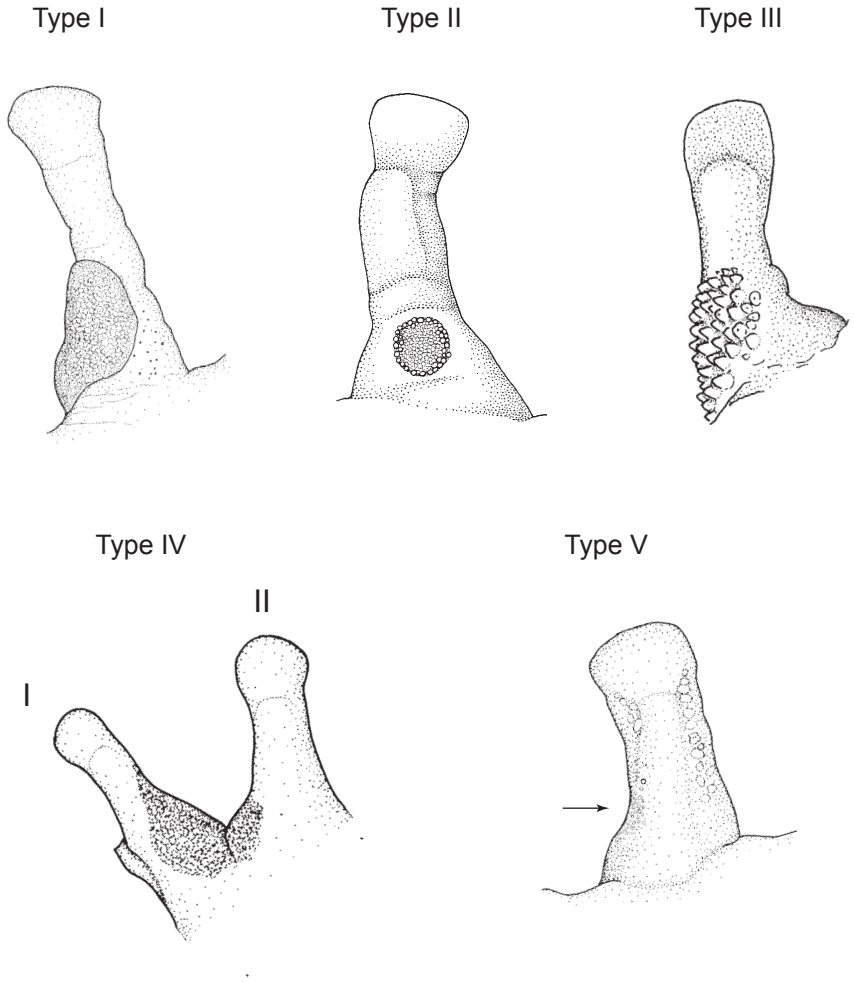

Fig. 2.4. Nuptial morphologies in Centrolenidae (sensu Cisneros-Heredia and McDiarmid, 2007). Arrow indicates glandular cluster typical in species of Hyalinobatrachium, as defined herein. Note that Morphology IV has only been reported in *Cochranella litoralis,* and differs from the description provided by Cisneros-Heredia and McDiarmid (2007). Species illustrated: Type I, *Cochranella posadae,* QCAZ 26023; Type II, *Centrolene lynchi,* MCZ 97846 (figure modified from Flores 1985); Type III, *Nymphargus armatus,* UVC 9400 (figure modified from Lynch and Ruiz-Carranza 1996); Type IV, *Cochranella litoralis,* ICN 13821; Type V, *Hyalinobatrachium aureoguttatum,* QCAZ 27429. Morphology VI is not illustrated.

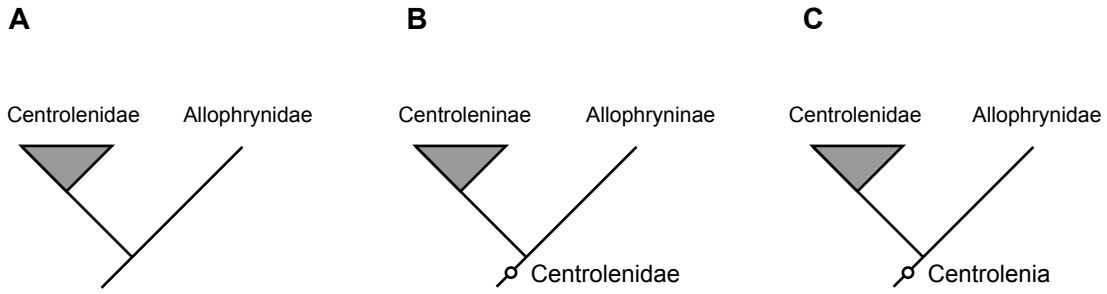

Fig. 2.5. Use of intermediate ranks as a mean to increase name stability. **(A)** Accepted relationships and taxonomy until 2006. **(B)** Proposal by Frost et al. (2006); note the change in names and species contents. **(C)** Recognition of Centrolenia as a Subsuperfamily; note that clade names and clade contents are maintained, and the Centrolenidae + Allophrynidae clade is formalized.

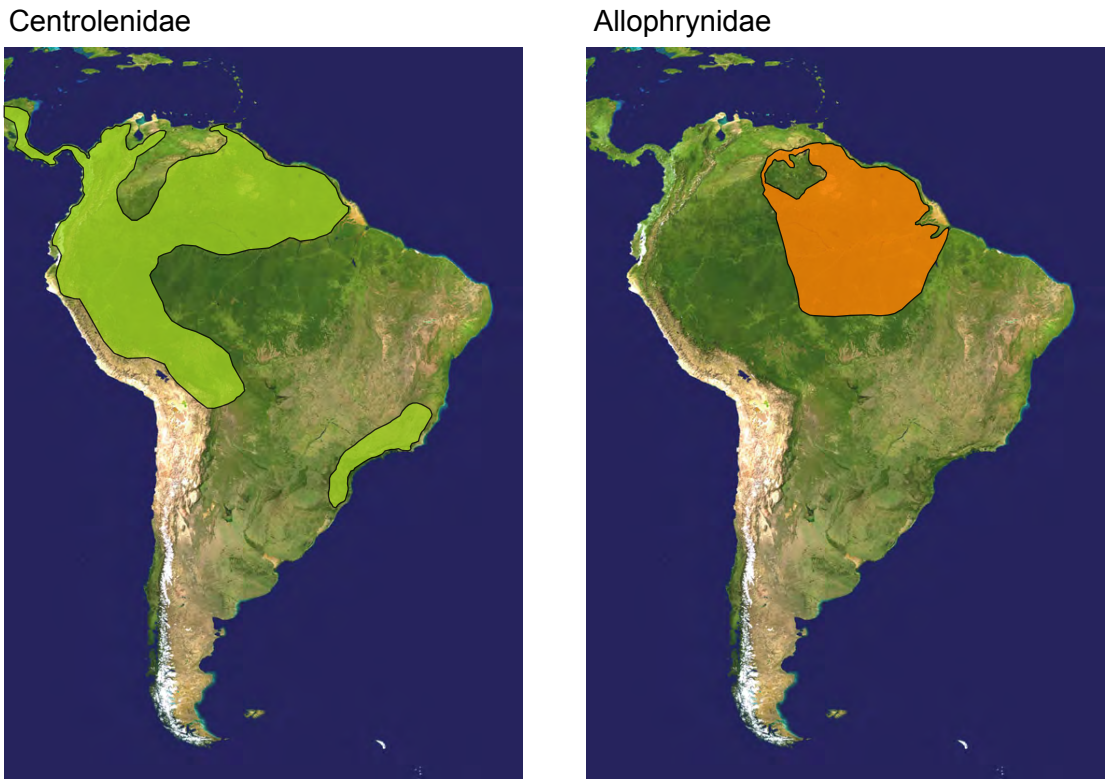

Fig. 2.6. Distribution of Centrolenidae and Allophrynidae. The distribution of Centrolenidae includes southern Mexico (not shown).

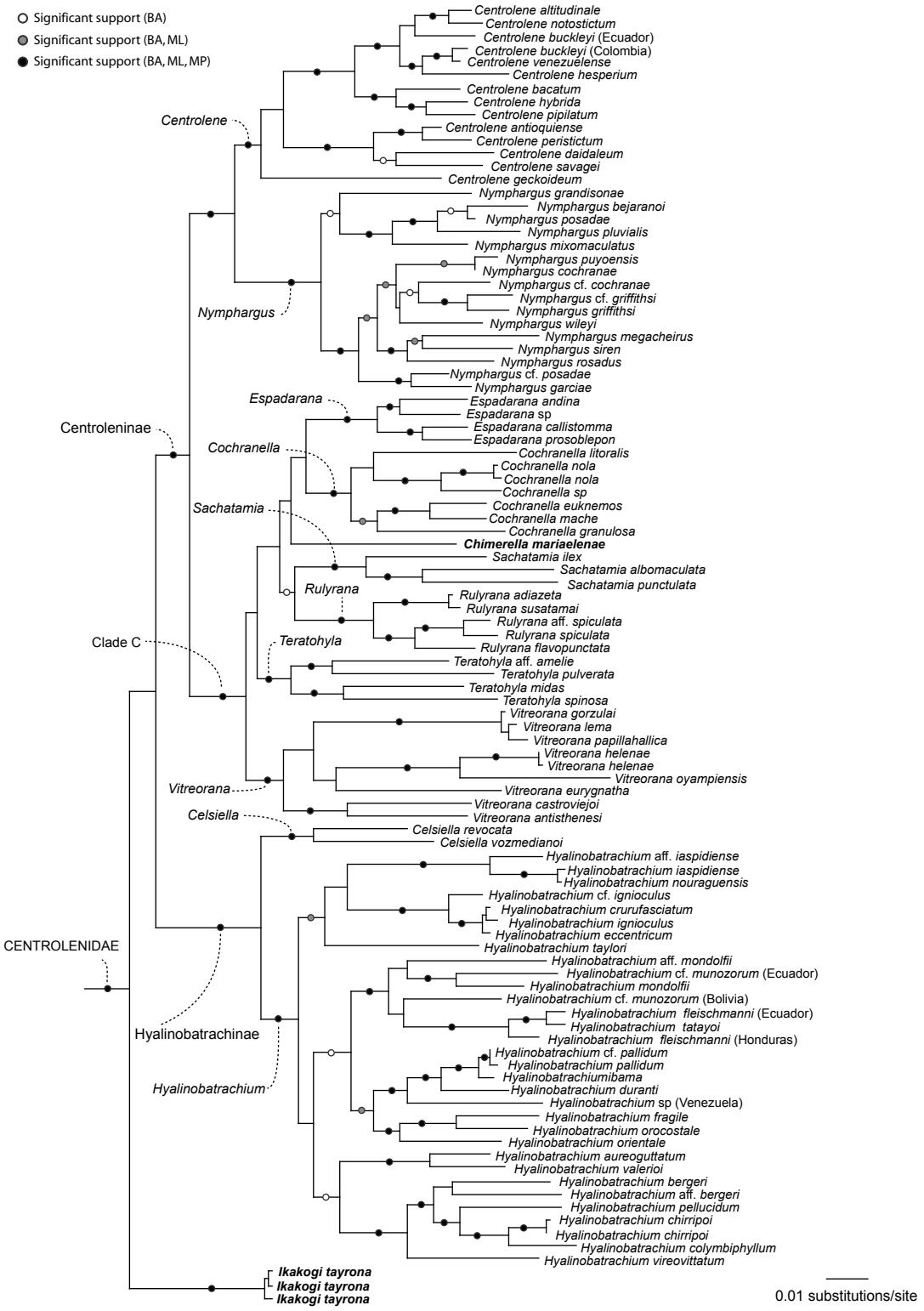

Fig. 2.7. Phylogenetic taxonomy of Glassfrogs. Topology was inferred from mitochondrial and nuclear genes using RAxML (see Chapter 1).

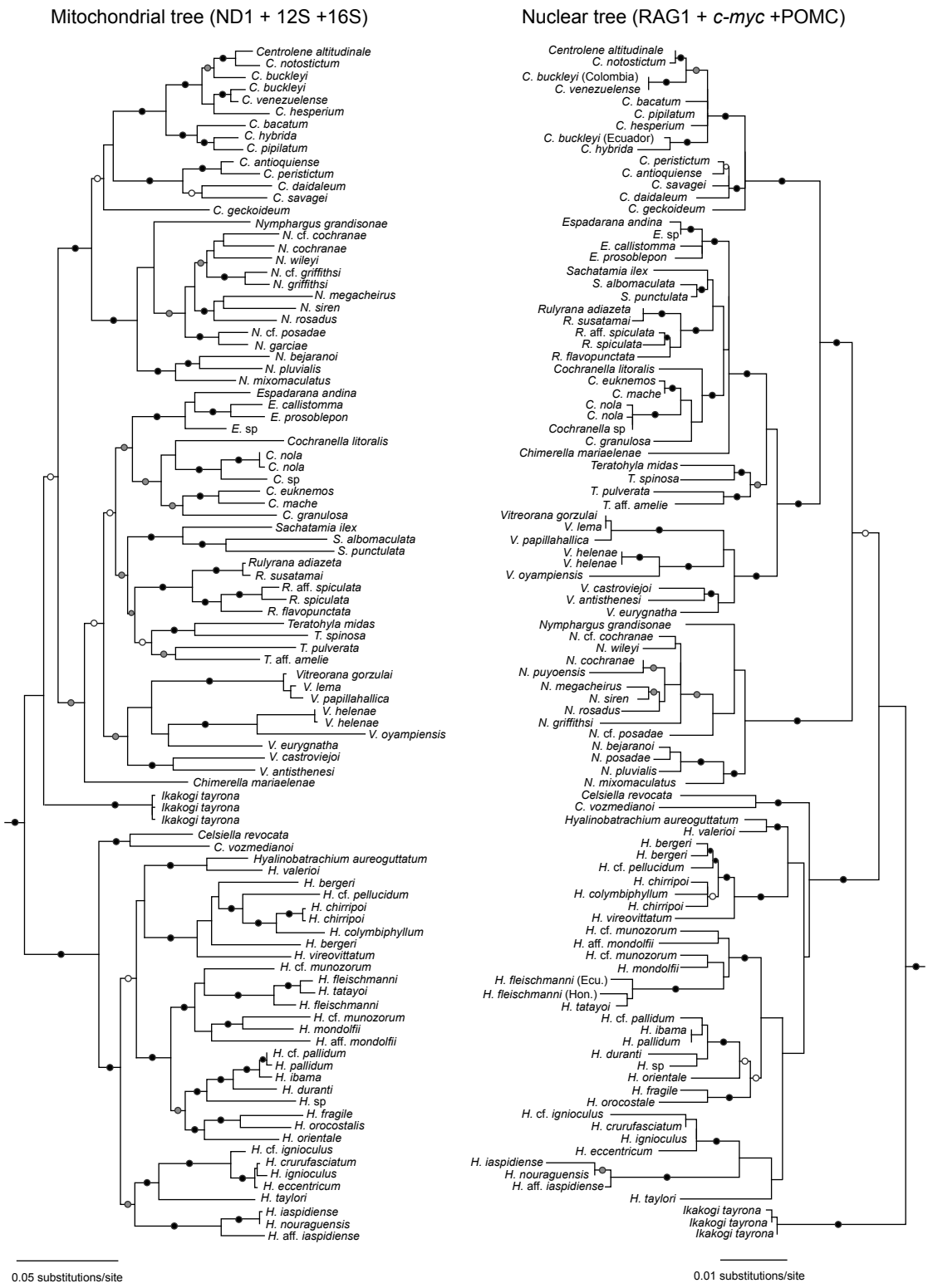

Fig. 2.8. Phylogeny of Glassfrogs inferred from mitochondrial (*left*) and nuclear (*right*) genes. Taxonomy as presented in Fig. 2.7.

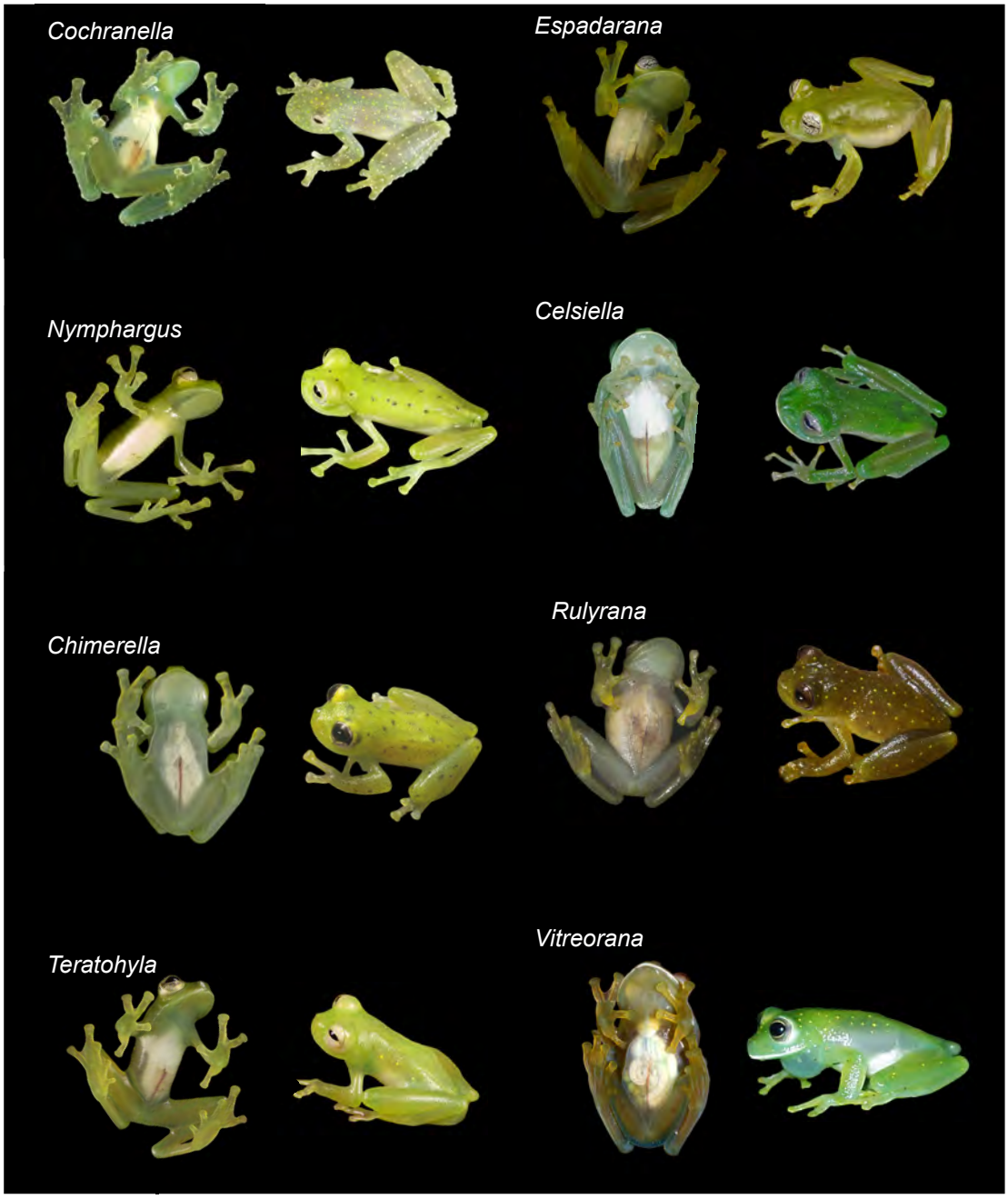

Fig. 2.9. Generic diversity within Centrolenidae. Illustrated species are: *Cochranella mache,* QCAZ 31327, SVL = 21.9 mm; *Espadarana callistomma,* QCAZ 28803, SVL = 28.7 mm; *Nymphargus cochranae,* QCAZ 31113, SVL = 30.3 mm; *Celsiella vozmedianoi,* MHNLS 17877, SVL = 28.7 mm; *Rulyrana flavopunctata,* QCAZ 32265, SVL = 27.5 mm; *Chimerella mariaelenae,* QCAZ 31729, SVL = 18.1 mm; *Teratohyla midas,* no number, SVL = ca. 20 mm; *Vitreorana antisthenesi,* MNHLS 17909, SVL = 24.4 mm

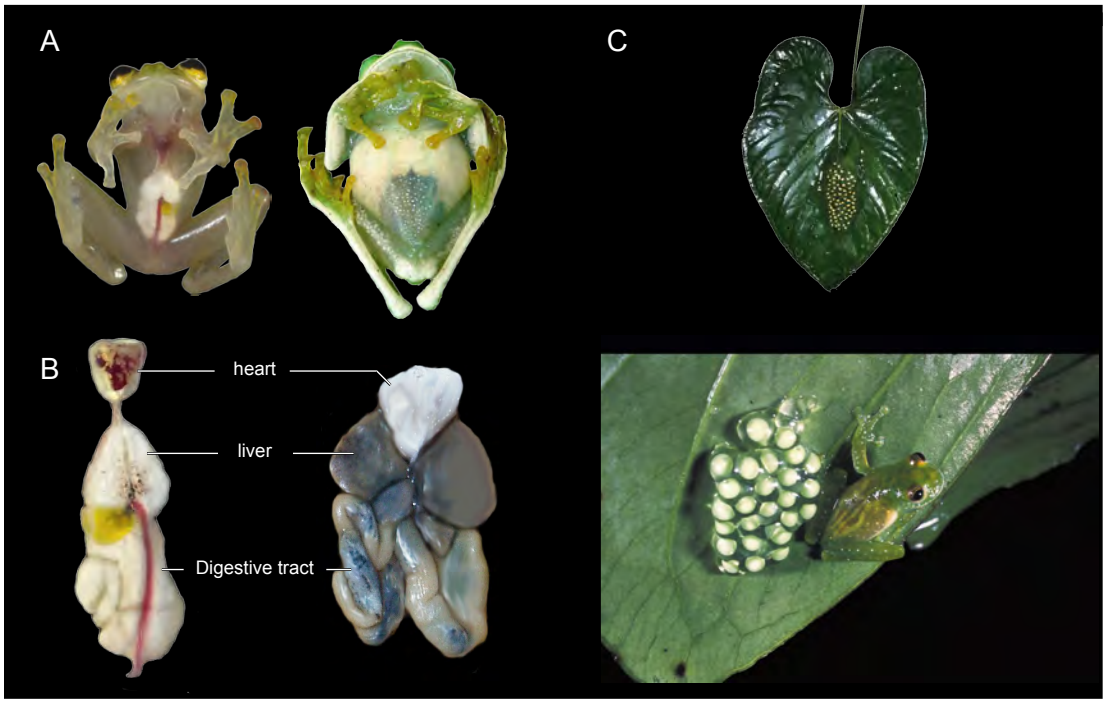

Fig. 3.1. Morphological and behavioral traits considered in this study. **(A)** Complete ventral transparency (left; *Hyalinobatrachium aureoguttatum*) and partial transparency (right; *Nymphargus posadae*). **(B)** Peritonea of heart, liver, and digestive tract covered by white iridophores (left; *H. aureoguttatum*); translucent peritonea on liver and digestive tract (right; *Centrolene buckleyi*). **(C)** Eggs deposited on the upper side of leaves (top; *Nymphargus grandisonae*) or on the underside of leaves (bottom; *Centrolene peristictum*). Note that there is a significant correlation between complete transparency of the ventral parietal peritoneum and the presence of iridophores on the hepatic and gastrointestinal peritonea as exemplified in *H. aureoguttatum* (A; left).

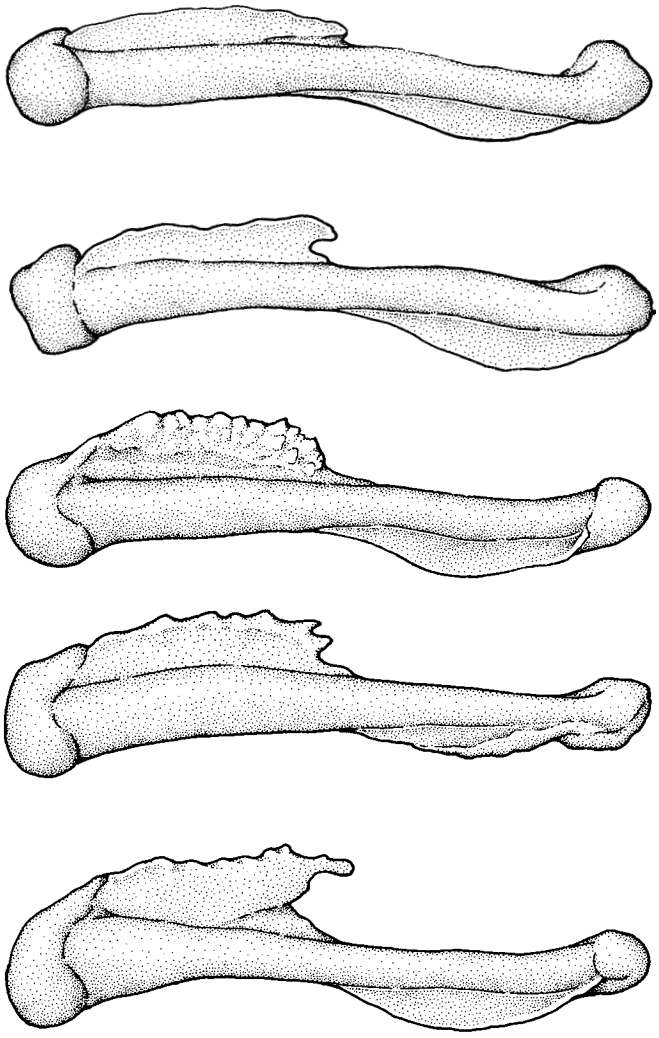

Fig. 3.2. Intraspecific variation of the crista ventralis in adult males of *Nymphargus griffithsi*. When the crista ventralis has a posterior extension (as shown in the illustration at the bottom), it is described as having a humeral spine. Specimens illustrated, from top to bottom, are KU 166325, 166323, 188148, 288991, 288992.

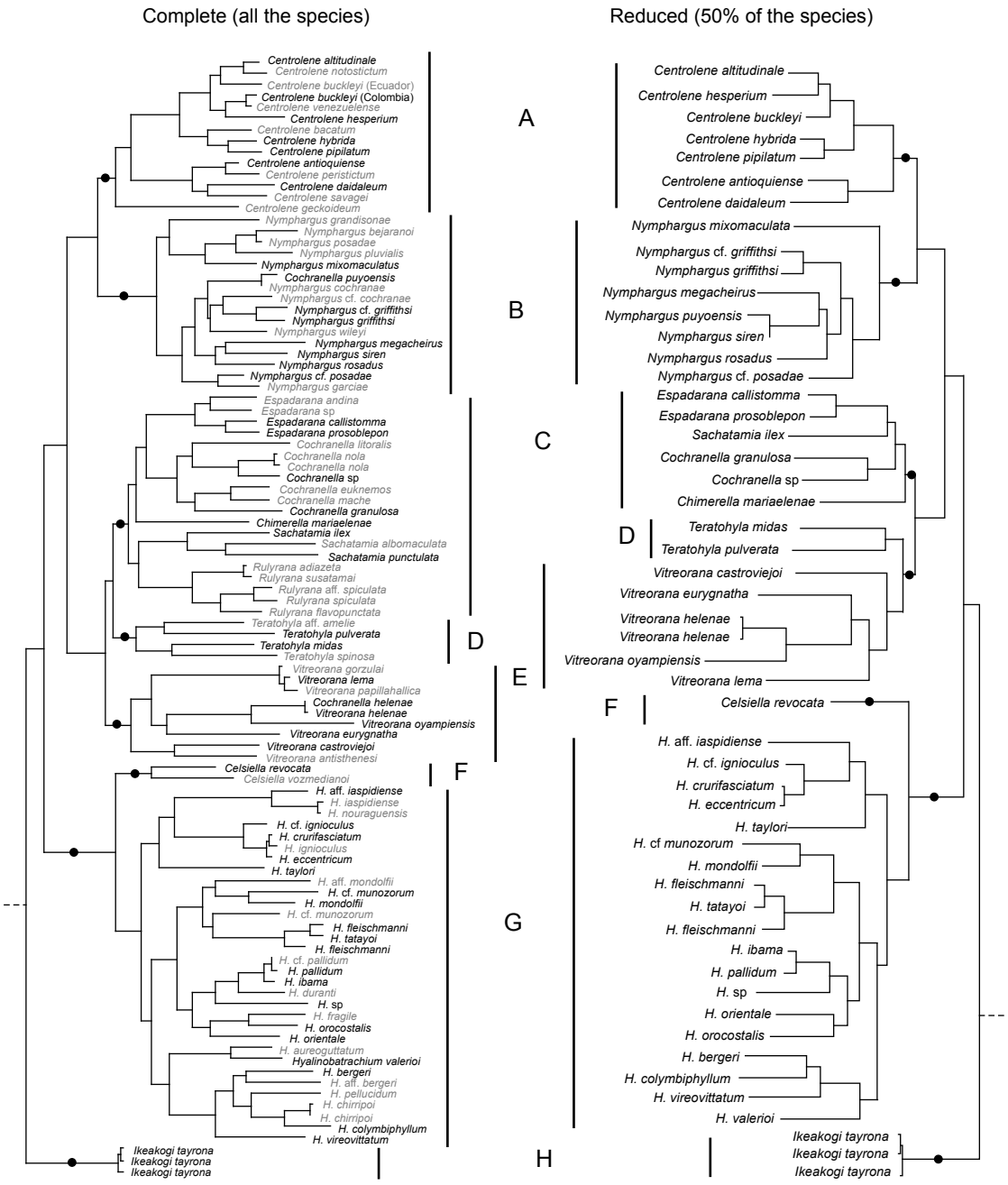

Fig. 3.3. Phylogeny of Glassfrogs with unreduced (*left*) and reduced (*right*) taxon sampling. Note congruence between the major clades (indicated by letters and black circles) in the two topologies.

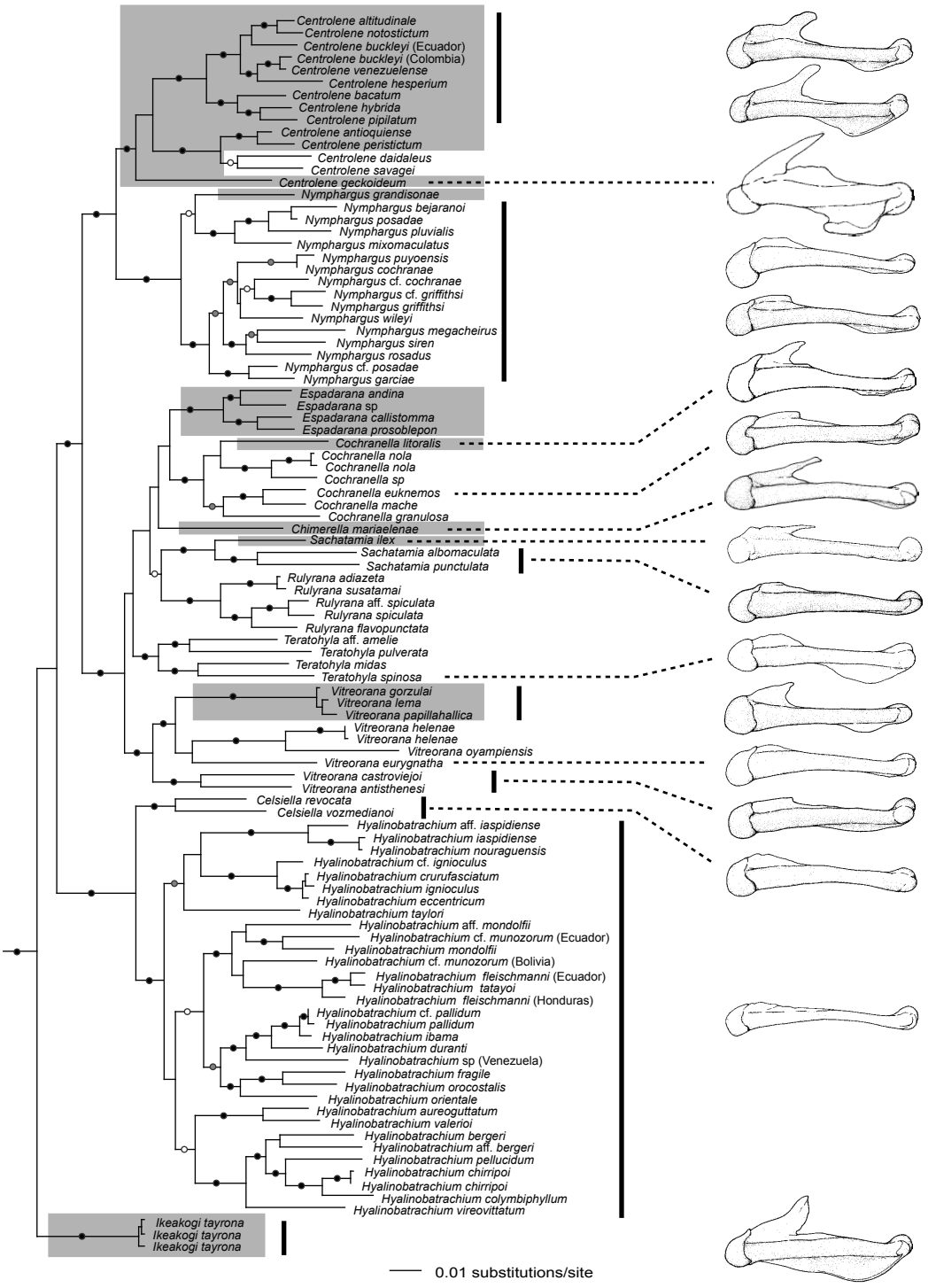

Fig. 3.4. Most likely reconstruction of the evolution of humeral spines (gray boxes) under the Mk1 model. Parsimony reconstruction provides several scenarios, with one being as shown in this figure. In contrast, the Asymmetric model significantly supports a single origin at the root and 13 subsequent losses (see Table 3.3.1). Circles represent nodal support (see Fig. 1.2).

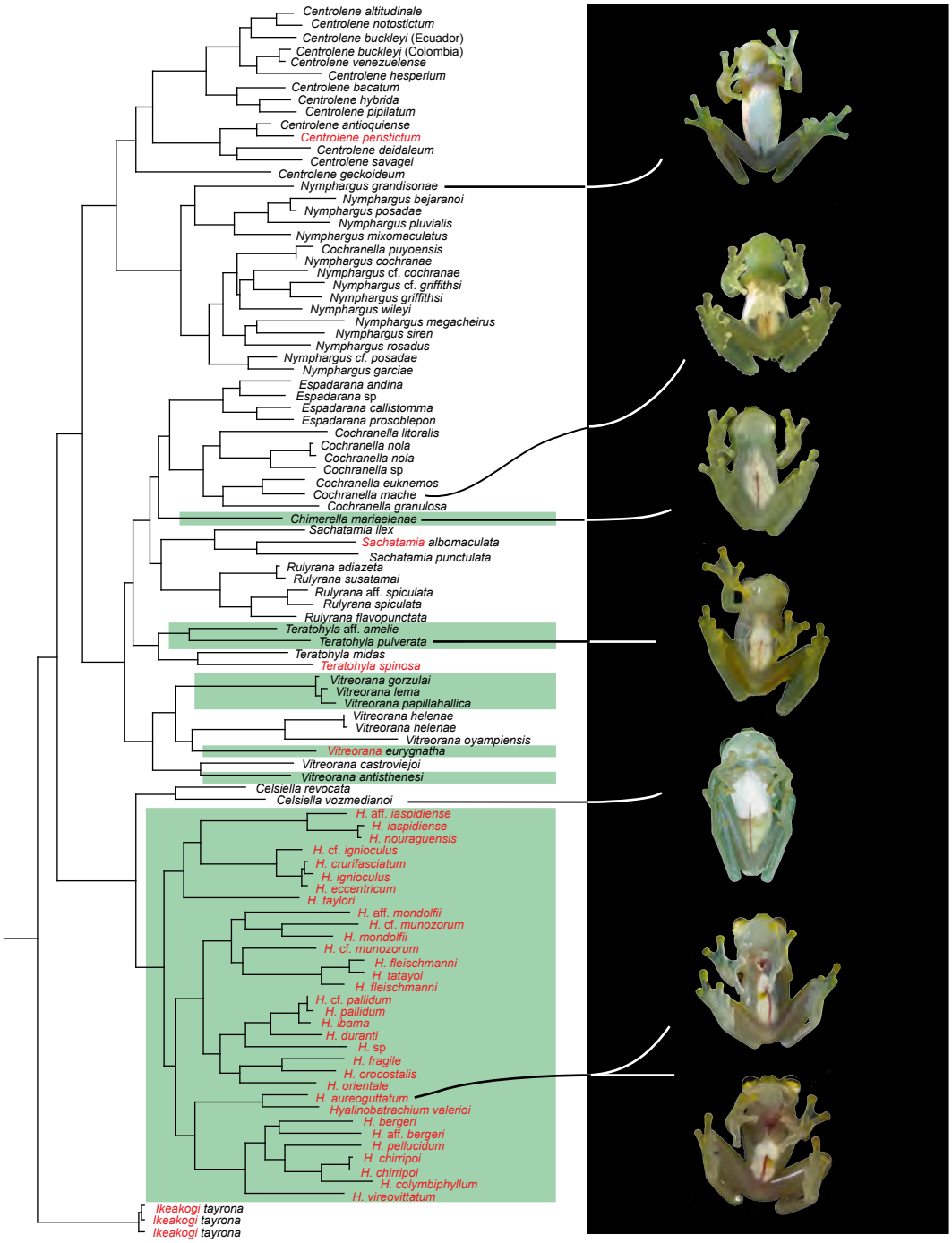

Fig. 3.5. Evolution of complete ventral transparency (green boxes) under the Mk1 and Asymmetrical models; the parsimony reconstructions provides several scenarios, one of which is congruent with that shown in the figure. The deposition of eggs on the underside of leaves (red labels) has evolved four independent times. Species that are polymorphic for the behavioral trait are indicated by red and black labeling. Ventral transparency is correlated with the presence of white iridophores on the hepatic and visceral peritonea (see text).

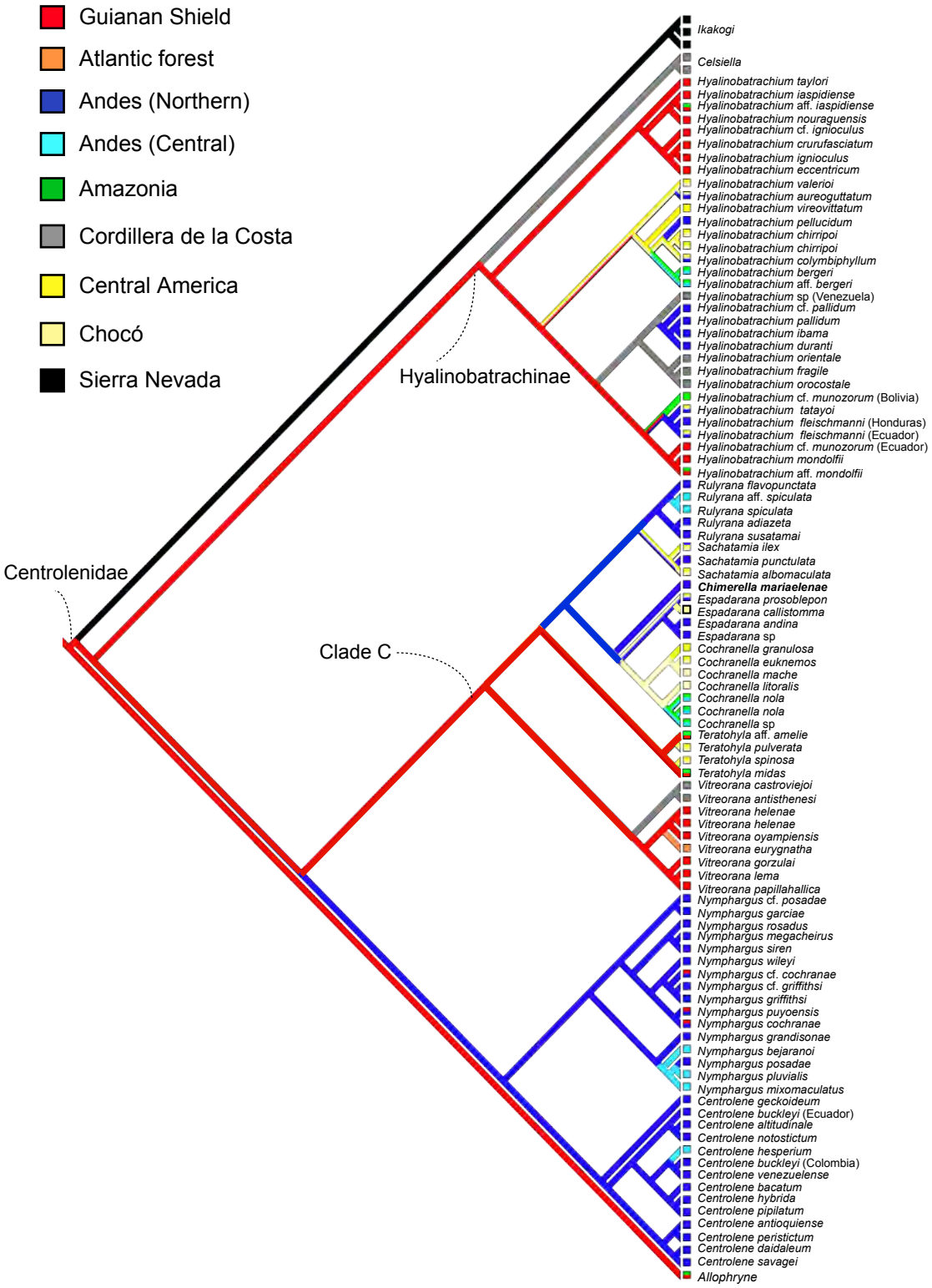

Fig. 4.1. Most parsimonious ancestral area reconstruction of Glassfrogs. As outgroup, the sister taxon of Centrolenidae, *Allophryne ruthveni,* was included in the analysis.

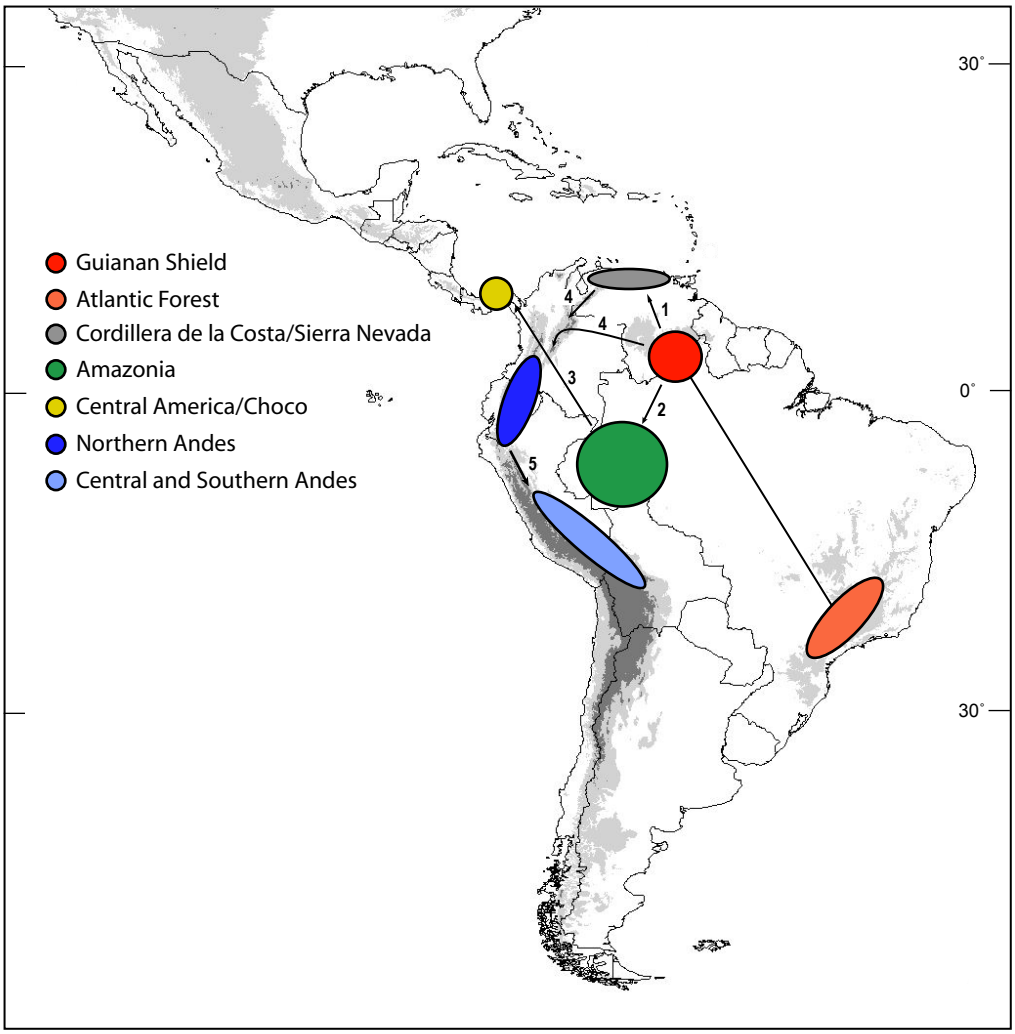

Fig. 4.2. Schematic scenario of the origin and main dispersal events of Glassfrogs. The most recent common ancestor of the group originated in the Guianan Shield and dispersed to Cordillera de la Costa, lowlands (Amazonia, Chocó, Central America), Northern Andes, and Southern Andes. Glassfrogs in the Atlantic Forest are hypothesized to be connected to Guianan species, from which were separated by a vicariant event (i.e., Amazon River).

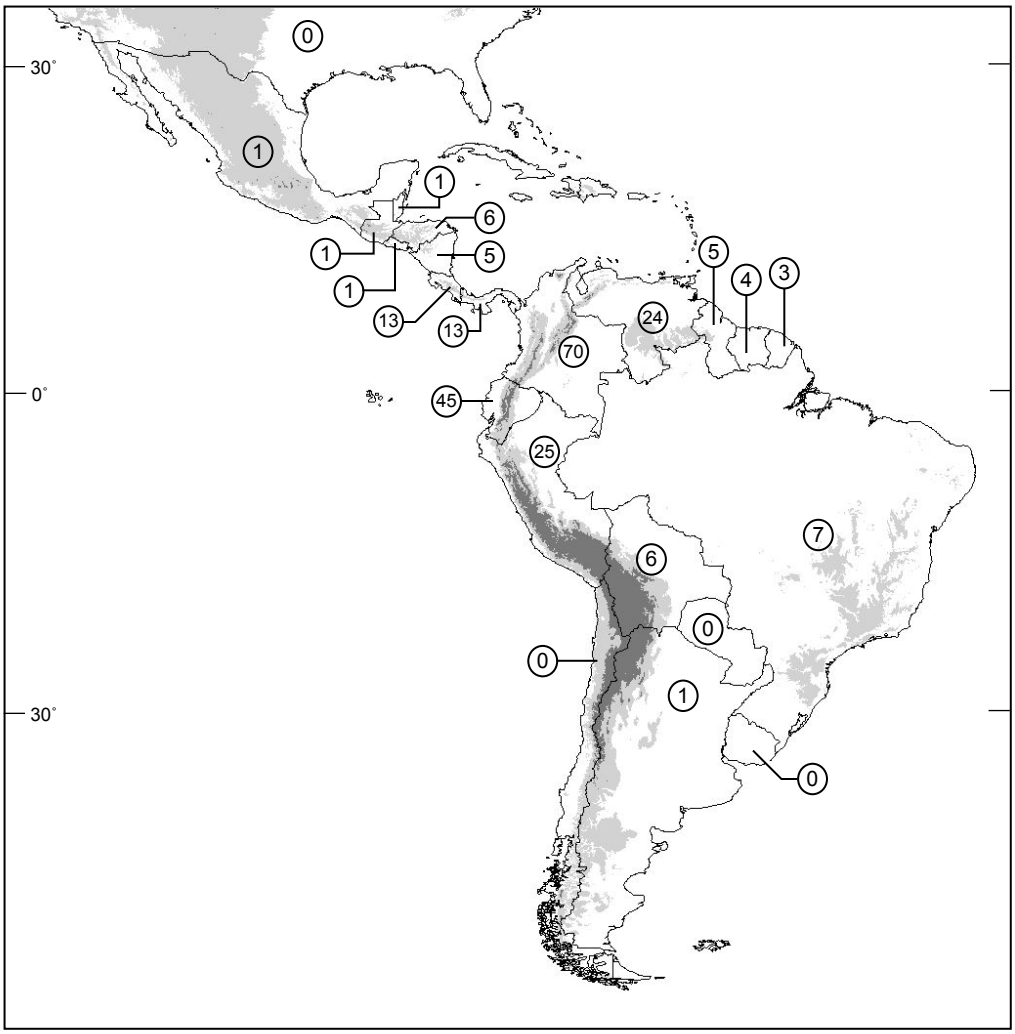

Fig. 4.3. Number of centrolenid species per country. Note that diversity decreases as latitude increases.